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Acute Effects of (Bis)tributyltin Oxide on Marine Organisms

Summary of Work Performed 1981 to 1983

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The acute effects of bis(tri-n-butyltin) oxide (TBTO) on six species of marine invertebrates were assessed to determine the relative toxicity of TBTO to a variety of organisms, and these results were compared with copper toxicity. Survival was the parameter measured for each species. Results indicated that TBTO was significantly more toxic than copper to all species by a factor of 10. In addition, organotin toxicity was roughly proportional to the number of butyl groups, i.e., monobutyltin less toxic than dibutyltin which was less toxic than tributyltin. The species tested fell into two categories: resistant and sensitive. The 96-hour LC_{50}s ranged between 15 and 30 parts per billion (ppb) TBTO for the resistant species, which included *Protothaca staminea*, *Mytilus edulis*, *Citharichthys stigmaeus*, and *Neanthes arenaceodentata*. The 96-hour LC_{50}s ranged between 1- and 2-ppb TBTO for the sensitive species, which included *Metamysidopsis elongata* and *Acartia tonsa*.

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SUMMARY

From 1981 to 1983, members of the Marine Sciences Division (Code 522) of the Naval Ocean Systems Center (NOSC) conducted several acute toxicity tests assessing tributyltin oxide (TBTO), the toxicant in organotin-based antifouling paints. The primary objective was to determine the relative toxicity of TBTO to a variety of organisms and to compare these results to copper toxicity. The data from these tests have been analyzed and compared with data from both concurrent and pursuant toxicity tests to help define the effect of organotin exposure on the marine environment.

The results of the tests conducted from 1981 to 1983 are summarized and presented in tables A and B. Only the 96-hour exposure data are reported to allow easy comparisons with other 96-hour data. When applicable, an LC_{50} value was estimated from these results. It should be emphasized that these are estimated values; only three concentrations were used per test.

In addition to performing these toxicity tests, a literature survey was undertaken for comparative and background purposes. The results of this search are presented as appendix A.

Table A. Summary results for the 96-hour exposures to organotins and copper. The type of test, data performed, toxicant concentration, 96-hour percent survival, and estimated LC_{50} values are presented. All concentrations are in parts-per-billion (ppb).

***Mytilus edulis* (Mussels)**

TBTO Test #1
4 7 81

Control:	100%
3.0:	100
15.0:	80
76.0:	10

***Protothaca staminea* (Clams)**

TBTO Test #1
1 14 81

Control:	100%
3.0:	100
15.0:	100
76.0:	100

TBTO Test #2
4 7 81

Control:	100%
40.0:	100
119.0:	100
271.0:	100

Copper Test #1
4 6 81

Control:	100%
250.0:	100
750.0:	97
2250.0:	97

***Citharichthys stigmaeus* (Fish)**

TBTO Test #1
4 6 81

Control:	80%
3.0:	87
19.0:	50
123.0:	0

Copper Test #1
4 6 81

Control:	80%
250.0:	47
750.0:	60
2250.0:	13

Table A. Summary results for the 96-hour exposures to organotins and copper (continued).

***Neanthes arenaceodentata* (Worms)**

TBTO Test #1 4/7/81		Copper Test #1 4/7/81		TBTO Test #2 7/27/81	
Control:	97%	Control:	97%	Control:	100%
10.0:	97	250.0:	60	4.0:	85
35.0:	0	750.0:	0	10.0:	0
150.0:	0	2250.0:	0	14.0:	0

***Metamysidopsis elongata* (Mysids)**

TBTO Test #1 1/12/81		TBTO Test #2 4/7/81		Copper Test #1 4/6/81	
Control:	97%	Control:	77%	Control:	77%
1.0:	97	2.0:	17	10.0:	77
3.0:	83	10.0:	0	30.0	13
14.0:	0	14.0:	0	90.0:	3

TBTO Test #3 7/27/81		TBTO Test #4 11/16/82		SPC-Leachate Test 11/16/82	
Control:	86%	Control:	90%	Control:	90%
0.2:	52	0.25:	86	0.25:	86
1.0:	12	1.0:	80	1.0:	82
3.0:	2	4.0:	16	4.0:	20
8.0:	0				

TBTO Test #5 2/10/81		TBTO Test #6 6/1/81	
Control:	96%	Control:	98%
1.0:	22	1.0:	90
4.0:	0	3.0:	8
22.0:	0	4.0:	0

MONOBUTYLTIN CHLORIDE 5/17/83		Test #7 DIBUTYLTIN CHLORIDE 5/17/83		TRIBUTYLTIN CHLORIDE 5/17/83	
Control:	71%	Control:	71%	Control:	71%
16.0:	62	2.0:	72	0.75:	60
161.0:	65	11.0:	68	1.5:	74
809.0:	67	56.0:	32	6.0:	19

***Acartia tonsa* (Copepods)**

TBTO Test #1 3/30/81		Copper Test #1 3/30/81	
Control:	63%	Control:	63%
1.0:	0	10.0:	36
3.0:	0	50.0:	26
15.0:	0	250.0:	0

Table B. Estimated LC_{50} values for TBTO and copper.

	TBTO	Copper
Mytilus edulis		
4-Day	35 ppb	NA*
10-Day	8	NA*
Protothaca staminea		
13-Day	110 ppb	
14-Day		250 ppb
Citharichthys stigmaeus		
4-Day	19 ppb	800 ppb
14-Day	7 ppb	250 ppb
Neanthes arenaceodentata		
4-Day (adults)	20 ppb	250 ppb
4-Day (juveniles)	7 ppb	
Metamysidopsis elongata		
4-Day	2 ppb	18 ppb
6-Day	1 ppb	
Acartia tonsa		
4-Day	$\ll$1 ppb	10 ppb

NA* = not available from results of these studies.

CONTENTS

INTRODUCTION

Although organotin-based antifouling (AF) coatings have been used on commercial and private ships since the early 1960s, little scientific information was available on their potential impact to the marine environment until the early 1980s. One reason for this is that the primary use of organotin compounds continues to be as a stabilizer for plastic polymers (PVC). As a result, the early research emphasized the human health aspects of using organotin compounds in industry. By 1976, world usage of organotin paints had increased to about 200 tons but this was still less than 1 percent of all organotin products in use. Even so, the main pathway of organotins to the marine environment is through organotin AF paints, and the amount being used is increasing annually. Prior to 1976, U.S. Navy usage of organotin paints was limited to coatings on submarine sonar domes and on Pacific Fleet submarine waterline areas (Bailey, 1984). The proposed Fleetwide use of organotin AF coatings by the U.S. Navy prompted closer scrutiny by U.S. regulatory agencies. Increased usage worldwide accelerated research on organotin toxicity to marine species during the last 7 years.

In 1981, the Naval Ocean Systems Center (NOSC) began a multiyear program to study the environmental impact of Fleetwide implementation. At that time there was a paucity of information on the fate and effect of these organotin compounds in the marine environment. Since then we have extensively studied the biology and chemistry of organotins in the marine environment. Environmental concentrations of organotins can now be determined with accuracy. Chemical analyses have reached a level of sophistication where organotins can be measured in the parts-per-trillion range, as well as determining the form of the alkyltins: monobutyltin, dibutyltin, or tributyltin. Unfortunately, the biological availability and environmental significance of these measurements remains unclear (Salazar 1986). Biological monitoring has advanced beyond the live-or-die criteria to the point of sublethal effects like enzyme systems (Pickwell and Steinert, 1984; Steinert and Pickwell, 1984), growth rates, and condition indices (Newton, Thum, Davidson, Valkirs, and Seligman, 1985; Valkirs, Davidson, and Seligman, 1985a). In addition, the standard static bioassay system has been upgraded with flowthrough capabilities that can be used for a variety of animals from clams to mysids (Meador, U'Ren, and Salazar, 1984).

The production and use of organotins in thermal stabilization has lead to a vast amount of literature on its chemistry and applications. Zuckerman, Reisdorf, Ellis, and Wilkinson (1978) present an extensive literature review which includes production, use, chemical characteristics, environmental fate, chemical and biological degradation, effects of exposure to organotin compounds and waste treatement. Stoner, Barnes, and Duff (1955), Barnes and Stoner (1958) and Noltes, Luijtan, and Van der Kerk (1961) also present their results of toxicity studies on a large number of organotin compounds. Although over 35 different organotin compounds were assessed, test animals were restricted to rats, mice, rabbits, guinea pigs, and fungi. A summary of the literature available prior to the start of these tests has been compiled and is presented as appendix A. In general, stress symptoms in fish were observed within 20 minutes after exposure to organotin concentrations greater then 0.10 parts per million (ppm), amphipods demonstrated a 13-percent survival rate at 10 parts per billion (ppb), while 100-percent larval lobster mortality was observed after a 24-hour exposure to 20-ppb organotin.

The work reported here includes the results of static acute bioassays performed from 1981 to 1983 as part of a Navy program to study the fate and effects of organotin antifouling coatings in the marine environment. In the first phase of testing, six faunal species were exposed to the chemical reagent tributyltin oxide (TBTO). For comparative

purposes, concurrent bioassays using copper as a toxicant were conducted and are also reported. Later phases included a static renewal test conducted in 1982, in which mysids were exposed to TBTO and a Self-Polishing Copolymer (SPC) leachate, and a test conducted in 1983 with mysids exposed to chemical reagents monobutyltin, dibutyltin, and tributyltin.

The main purpose of this report is to document research conducted at NOSC between 1981 and 1983 and not to review all the work conducted since. It should be clearly remembered that our early work showed trends and general toxicity only and few definite conclusions. Preliminary testing indicated organotins had the potential for a significant adverse environmental impact, but substantial scientific evidence to support this concept continues to be lacking. Since the Navy is considering Fleetwide implementation of organotin AF coatings, it is responsible for obtaining the best possible environmental data to justify such actions and documenting all previous work. Our early work was not nearly as sophisticated or definitive as our later work; however, documentation is necessary to assist future research on organotins and to supplement the Environmental Impact Statement now being prepared for the U.S. Navy. Although we do not believe these early data can be used to predict environmental impact and establish regulatory criteria, they may assist in establishing regulations. These data must be interpreted with caution (Salazar, 1986).

MATERIALS AND METHODS

SELECTION OF ORGANISMS

To represent the potential impact of organotins on the marine environment, six different marine species were exposed to TBTO. Species used were *Mytilus edulis* (mussel), *Protothaca staminea* (clam), *Citharichthys stigmaeus* (flatfish), *Neanthes arenaceodentata* (polychaete worm), *Metamysidopsis elongata* (mysid), and *Acartia tonsa* (copepod). All of these species are on the list of approved animals for dredge material bioassays compiled by the Environmental Protection Agency and the Army Corps of Engineers (EPA/COE, 1977). Many are routinely used for other bioassays as well, and comparative information for other toxicants is available. Although most of these animals lack sensitivity to toxicants, they were used primarily to establish baseline organotin toxicity levels. In addition, they were used to rank the toxicity of organotins against other toxicants. Further, we had used all of them in many previous bioassays and were familiar with maintaining and testing these animals.

The mussel *M. edulis* is a filter-feeding bivalve with a cosmopolitan distribution. Mussels are not very sensitive to many contaminants but they are nevertheless effective sentinels. They are one of the most widely used test animals in the world because of their distribution, ease of maintenance, and the representative nature of their filter-feeding mechanisms. Mussel watch programs to monitor tissue contaminant levels have been developed in many parts of the world, and we have used them for sublethal stress indicators (Pickwell and Steinert, 1984; Steinert and Pickwell, 1984).

The clam *P. staminea* is one of the most abundant on the west coast of North America. It has been routinely used for sediment bioassays because of its feeding modes and habitat even though not highly sensitive. Comparative information for other toxicants is available. *P. staminea* is a filter feeder like *M. edulis* but its habitat is the sediment rather than the water column. Both *M. edulis* and *P. staminea* are collected recreationally for consumption. Thus, it is necessary to determine the effect of organotins on such economically important species.

The benthic flatfish *C. stigmaeus* is very common along the west coast of North America and is one of the most prevalent in southern California coastal waters found at depths from 10 to 1200 feet. *C. stigmaeus* is not highly sensitive but is routinely used in many different types of bioassays because it is representative of the local bottom fish. Again, much literature exists on the effects of toxic substances on *C. stigmaeus*.

The polychaete worm *N. arenaceodentata* was used for a variety of reasons. It is readily available in laboratory culture, easily maintained in laboratory tests with or without sediment, and is a sediment-dwelling deposit feeder. A large toxicity literature database is available on this animal.

Plankton were represented by two species. The first was the mysid *M. elongata*, a hypoplanktonic crustacean that occurs in swarms just above the sandy bottom in depths up to 50 m. It lives in close association with the sediment during the day and migrates to the water surface at night (Mauchline, 1980). For this reason it can be considered to represent both near bottom and water column habitats. The mysid is the only test animal required in all dredge material bioassays throughout the U.S. We feel it is the most sensitive, representative, and reliable test animal we have ever used in the laboratory.

The second planktonic species we used was the copepod *Acartia tonsa*. This copepod is very common in southern Californian coastal waters and is routinely used in toxicity tests. Copepods are probably more sensitive than mysids but test results are not as

reliable when using field-caught animals (Sosnowski, Germund, and Gentile, 1979). Field-caught animals were used in the majority of mysid tests and in all copepod tests. Larval and juvenile mysid tests used laboratory-hatched animals.

EXPERIMENTAL CONDITIONS

All organotin bioassays were conducted under static conditions using coarsely filtered seawater. Natural seawater for these tests was pumped from approximately 250 meters offshore and filtered through sand. All organotin bioassays were performed at the Marine Sciences Laboratory Facility at NOSC. Water temperature was maintained between 13 and 14°C in a temperature and light controlled bioassay room (14L: 10D). Physical and chemical parameters of this seawater were measured daily with a Horiba U-7 water checker. Salinity ranged between 33 and 34 ppt, dissolved oxygen between 7.5 and 8.0 ppm, and pH between 7.7 and 8.1.

The conditions for all organotin tests are summarized in table 21. In most cases the organotin toxicant was added as the chemical reagent TBTO. In one test, monobutyltin, dibutyltin, and tributyltin were also added as chemical reagents. In another test, the toxicant was added as a lechate from painted panels, and toxicity was compared with the chemical reagent.

With the exception of clams and mussels, all animals were held in either polycarbonate (16 L) or Pyrex glass (1 L and 400 ml) test containers as these materials have demonstrated minimal organotin adsorption (Dooley and Homer, 1983). Clams and mussels were held in standard 10-gal glass aquaria. Aquaria and Pyrex beakers were nitric acid washed and then soaked in seawater for 72 hours prior to the start of each test. In most tests three replicates were used for each treatment and control condition; however, five replicates were sometimes used. The majority of replicates consisted of 10 animals each but in some tests the number of animals/replicate varied from 9 to 12.

All tests were static except mysid test #4. It was the only one with static-renewal conditions where test solutions were changed every 24 hours during the 4-day test. All tests were originally designed as 96-hour tests, but if survival was high after 96 hours, test duration was increased. Thus, tests reported here varied from 4 to 14 days. All animals except copepods were aerated during these tests. Clams, mussels, and fish were vigorously aerated at a rate between 500 and 1300 ml/minute. Worms and mysids received moderate aeration at 3 to 7 ml/minute. Only mysids were fed during these tests. Each mysid was fed 20 to 30 brine shrimp nauplii per day.

PREPARATION OF TEST SOLUTIONS

Copper and organotin stock solutions were prepared from chemical reagents. They were prepared just prior to the start of each test to decrease avaliable time for toxicant adsorption from solution onto the walls of the storage containers. Some measurements of actual organotin test concentrations were made during each test but they are not available for each day at each concentration.

The TBTO stock was prepared by adding 1 ml of TBTO reagent to 1 liter of filtered seawater. The solution was placed on a rotary shaker for 48 to 72 hours and then filtered through a double layer of pre-moistened filter paper. After filtering, the TBTO concentration of the stock was measured with a graphite furnace atomic absorption spectrophotometer (GF-AAS) (Valkirs, Seligman, Vafa, Stang, Homer, and Lieberman, 1985b).

The copper stock was prepared by adding appropriate amounts of $CuCl_2$ to Milli-Q deionized water to provide a stock concentration of 1000 ppm which was measured by GFAAS. Test concentrations were made by diluting an aliquot of stock solution with seawater.

Two different solutions were prepared for the TBTO leachate test performed with mysids (mysid test #4). The TBTO reagent solution was prepared as indicated above for organotin stock solutions. The leachate solution was prepared by rotating 15-cm SPC-coated discs in seawater for 12 hours after which an aliquot of leachate was withdrawn and measured by GF-AAS. This solution was diluted with filtered seawater to obtain desired concentrations.

Solutions for the mono-, di-, and tributyltin tests with mysids were prepared from the appropriate reagents as per the methods for TBTO stock solution preparation.

RESULTS

MYTILUS EDULIS (MUSSELS)

Mussels were exposed to TBTO concentrations of 3, 15, and 76 ppb in three replicates of 10 animals each for 10 days in January 1981. Mussel survival in the controls and the 3-ppb TBTO treatment remained at 100 percent for the entire 10-day test. After 4 days, mussel survival in 15- and 76-ppb TBTO treatments was 80 and 10 percent respectively (table 1, figure 1*). Survival in these treatments dropped to 50 and 0 percent after 10 days.

Seawater samples were collected daily from the 15- and 76-ppb treatment tanks during the 96-hour test. TBTO concentrations decreased after 24 hours to 13.0 and 52.0 ppb respectively (table 22). The TBTO concentration in a chemical blank tank containing no animals dropped from 76 to 39 ppb over the 4-day period. These results indicate a significant uptake of TBTO by mussels and the tank itself. From these data, an estimated 10-day LC_{50} for *M. edulis* is about 8.0-ppb TBTO.

PROTOTHACA STAMINEA (CLAMS)

Clams were exposed to TBTO concentrations ranging from 3 to 271 ppb in two separate tests. In January 1981, clams were exposed to TBTO concentrations of 3, 15, and 76 ppb in three replicates of 10 animals each for a 10-day period. This test was concurrent with the mussel test previously described. Survival was 100 percent in all test conditions after the first 4 days. Survival for controls and the 15-ppb treatments remained at 100 percent for the entire 10-day period. Survival in the 3- and 76-ppb treatments was 97 and 80 percent respectively after 10 days (table 2, figure 2).

Test solutions were analyzed at the start of the test to determine actual TBTO concentrations being used. No other chemical measurements were made during this particular test. It was assumed that test concentrations in the clam tanks would be similar to test concentrations in the mussel tanks.

In April 1981, clams were exposed to TBTO concentrations of 40, 119, and 271 ppb with three replicates of 10 animals each for 13 days. Again, 100-percent survival was observed in all treatments and controls after 4 days (table 3, figure 3). Control survival dropped to 97 percent on day 6 and remained there until the end of the 13-day test. Survival in all treatment tanks decreased after 7 days' exposure with 13-day survival rates of 77, 23, and 27 percent for the 40-, 119-, and 271-ppb TBTO exposures respectively.

Chemical measurements were made during this second test to determine the actual concentration of TBTO in solution over time (table 22). After 4 days, TBTO in the nominal 271-ppb treatment decreased to 105 ppb, less than half of the initial concentration. Further, the concentration continued to decrease during the 13-day test period to 72 ppb. TBTO in the nominal 119-ppb treatments decreased to 59 ppb after 24 hours and to 6 ppb after 13 days. TBTO in the nominal 40-ppb treatment decreased to 2 ppb after 13 days.

For comparative purposes, clams were exposed to copper (Cu) concentrations of 250, 750, and 2250 ppb in three replicates of 10 animals each for 14 days during April 1981 (table 4, figure 4). Clams exposed to 750- and 2250-ppb Cu exhibited only 3-percent mortality after 4 days. Control survival dropped to 97 percent by day 7, where it remained for the duration of the 14-day test. By the end of the test treatment, survival was 53, 40, and 27 percent for the 250-, 750-, and 2250-ppb exposures respectively. Cu concentrations were not measured in test tanks during the exposure period.

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*Tables and figures are placed at the end of the text, before appendix A.

Considering the decreasing concentration of TBTO during the study, the 13-day LC_{50} for *P. staminea* estimated from results in this study is probably between 100- and 120-ppb TBTO. This can be compared to a 14-day LC_{50} for Cu of 250 ppb. Ten-day assessments resulted in minor effects at the 76.0-ppb exposure while 13-day assessments resulted in decreased survival at 40.0-ppb TBTO.

CITHARICHTHYS STIGMAEUS (FLATFISH)

C. stigmaeus were exposed to 3-, 19-, and 123-ppb TBTO in three replicates of 10 animals each for 14 days during April 1981. After 4 days, fish survival in control tanks was relatively low at 80 percent, 87 percent in the 3-ppb TBTO treatment, and 47 percent in the 20-ppb treatment. All the fish in the 120-ppb treatment died within 48 hours. By the end of the 14-day test, control survival had dropped to 70 percent. Survival in the 3- and 19-ppb treatments also dropped to 60 and 43 percent respectively (table 5, figure 5).

TBTO concentration in the 3- and 20-ppb treatments decreased to the detection limit of 2 ppb after 4 days (table 22), and TBTO in the 120-ppb treatment decreased to 12 ppb during the same period.

A 14-day Cu toxicity test was also performed in April 1981 assessing concentrations of 250-, 750-, and 2250-ppm Cu with three replicates of 10 animals each. Substantial mortality was observed in the 250- and 750-ppb treatments withing 24 hours. After 96 hours, mortality had reached 80, 47, 60, and 13 percent in the control 250-, 750-, and 2250-ppb treatments respectively. By the end of the test, control survival was 70 percent, while survival in the 250-, 750-, and 2250-ppb Cu treatments was 23, 0, and 0 percent respectively (table 6, figure 6).

We estimate the 96-hour LC_{50} for *C. stigmaeus* to be about 19.0-ppb TBTO. However, 14-day survival in the same treatment was 43 percent, 7 percent lower. Thus, the 14-day LC_{50} may be in the 5.0 to 9.0-ppb TBTO range. For comparative purposes, the 14-day LC_{50} for *C. stigmaeus* exposed to Cu is near 250 ppb.

NEANTHES ARENACEODENTATA (POLYCHAETE WORMS)

Adult polychaete worms were exposed to TBTO concentrations of 10, 35, and 150 ppb with three replicates of 10 animals each for 6 days during April 1981. After 6 days, survival in the controls and 10-ppb treatment was 97 and 93 percent respectively (table 7, figure 7). Survival in the 35-ppb tanks rapidly dropped to 27 percent after 2 days and to 0 percent after 3 days. Exposure to 150 ppb, the highest concentration tested, resulted in complete mortality after 24 hours. The TBTO concentrations were measured in the 40- and 150-ppb treatments only. After 4 days the TBTO concentration decreased to 7 ppb in the 40-ppb treatment and to 80 ppb in the 150-ppb treatment. TBTO concentrations are given in table 22.

In conjuction with the TBTO test, adult worms were exposed to Cu concentrations of 250, 750, and 2250 ppb in three replicates of 10 animals each for 6 days. Ater 48 hours, survival was 100, 100, 13, and 0 percent for the control 250-, 750-, and 2250-ppb treatments (table 8, figure 8). By day 4, mortality at Cu concentrations greater than 250 ppb was 100 percent. At this time, control survival declined slightly to 97 percent, and survival in the 250-ppb treatment decreased to 50 percent. By day 6, survival at all Cu concentrations was 0 percent; control survival reamained at 97 percent.

Juvenile worms were used in a comprehensive 4-day test during July 1981. TBTO concentrations of 4, 10, 14, and 20 ppb were used with five replicates of 10 animals per treatment. Water samples were collected daily for TBTO measurements. To reduce the

amount of water withdrawn per tank and to provide a more representative sample, subsamples from each tank were combined. Control survival was 100 percent for the entire 4 days, while juvenile worms in the 4-ppb TBTO treatment tanks had an 85 percent survival rate. Survival in the 10-ppb treatment dropped to 57 percent after 2 days and 0 percent after 4 days. Similarly, juvenile worm survival in the 14-ppb treatment dropped to 10 percent after 2 days and to 0 percent after 4 days. In tanks containing 20 ppb-TBTO, juvenile worm survival was 0 percent after 2 days (table 9, figure 9).

As with all previous static bioassays, the concentration of TBTO in solution decreased dramatically during the juvenile worm test (table 22). TBTO in the two low-concentration during the 4-day test; the 4-ppb solution decreased to 2 ppb, the detection limit of the analytical method, and the 10 ppb solution decreased to 5 ppb. After 4 days, measurable TBTO decreased to 9 and 13 ppb in solutions originally prepared at 14 and 20 ppb respectively.

The estimated 96-hour LC_{50} for adult *N. arenaceodentata* is close to 20.0-ppb TBTO. For comparative purposes the Cu data show an estimated 96-hour LC_{50} of 250.0 ppb. Similar tests conducted with juvenile *N. arenaceodentata* resulted in estimated 96-hour LC_{50} values of 7.0-ppb TBTO.

METAMYSIDOPSIS ELONGATA (MYSIDS)

Since our previous dredge material bioassays had shown that mysids are highly sensitive yet reliable test animals, mysid bioassays with TBTO were emphasized. We conducted seven separate sets of tests with *M. elongata* at TBTO concentrations ranging from 0.20 to 22.0 ppb. For comparative purposes, one Cu experiment was run concurrently with the second TBTO experiment. Cu concentrations ranged between 10.0 and 90.0 ppb.

Test #1: January 1981

In the first test, adult mysids were exposed to TBTO concentrations of 1.0, 3.0, and 15.0 ppb in three replicates of 10 animals each for 7 days. After 4 days, control survival was 97 percent. Survival in the 1.0-, 3.0-, and 15.0-ppb TBTO treatments were 97, 83, and 0 percent respectively. Control survival remained at 97 percent through day 7; treatment survival dropped to 93, 33, and 0 percent at 1.0-, 3.0-, and 15.0-ppb TBTO respectively (table 10, figure 10). TBTO concentrations in seawater taken from the nominal 15.0-ppb tanks were measured on days 1 and 4 of the test. The TBTO concentration in samples collected on day 1 dropped to 9.0 ppb and declined to 4.0 ppb over the next 3 days (table 22).

Test #2: April 1981

The second series of tests with mysids consisted of Cu and organotin assessments run concurrently. Nominal TBTO concentrations were 2.0, 10.0, and 14.0 ppb. Nominal Cu concentrations were 10.0, 30.0, and 90.0 ppb. Three replicates of 10 animals each were used for each treatment in addition to a set of controls.

Results of the TBTO study are presented in table 11 and figure 11. Control survival was poor, dropping to 77 percent after 4 days and to 47 percent the following day. Complete mortality was observed in the 14.0-ppb TBTO treatment after 2 days and in the 10.0-ppb exposure after 3 days. Survival at the lowest concentration of 2.0 ppb was 3 percent after 5 days. Chemical analyses of seawater samples taken from the nominal 14.0-ppb tanks on day 4 indicated TBTO concentrations declined to 6 ppb. The TBTO concentration in the nominal 10.0-ppb tanks dropped to 9.0 ppb after 4 days (table 22).

One set of mysid controls was used for both the Cu and TBTO tests started on 7 April 1981. Therefore, control survival for the Cu experiment was the same at 77 percent after 4 days and 47 percent after 5 days. Survival in the 10.0-ppb Cu treatment was 77 percent after 4 days and 40 percent after the fifth day. Survival was 13 and 0 percent in the 30.0- and 90.0-ppb Cu treatments respectively after 5 days (table 12, figure 12). The Cu test was allowed to run for 7 days to determine the difference between the control and the 10-ppb treatment after a longer exposure period.

Test #3: July 1981

TBTO concentrations of 0.2, 1.0, 3.0, and 8.0 ppb were used in the third test with adult mysids. The test was run for 4 days. There were five replicates per treatment, with the number per replicate varying between 9 and 12. After 4 days, control survival was 86 percent while treatment survival was 52, 12, 2, and 0 percent at 0.2-, 1.0-, 3.0-, and 8.0-ppb TBTO respectively (table 13, figure 13).

Test #4: November 1982

In this 6-day test, adult mysids were exposed to TBTO chemical reagents and TBTO paint leachates at nominal concentrations of 0.25, 1.0, and 4.0 ppb with five replicates of 10 animals each. TBTO solutions were prepared from the reagent as previously described. TBTO leachate water was prepared by soaking panels coated with SPC, an organotin-based AF paint, in seawater. Unlike all previous tests, this was a static renewal experiment where dead animals were removed and fresh toxicant and seawater added daily. Five replicates per treatment with 10 organisms per replicate were used. The same control was used for both the reagent and leachate assessments. Control survival was very good, dropping to 90 percent after 3 days and remaining there until the 6-day experiment was terminated.

Mysids exposed to the two types of TBTO toxicant demonstrated similar reponses in survival (tables 14 and 15, figures 14 and 15). After 4 days, survival of mysids exposed to the TBTO reagent was 86, 80, and 16 percent at the nominal 0.25-, 1.0-, and 4.0-ppb treatments respectively. After 6 days, treatment survival decreased to 80, 58, and 2 percent for the same respective treatments. In the SPC leachate series, 4-day survival was 86, 82, and 20 percent at the 0.25-, 1.0-, and 4.0-ppb exposures respectively. After 6 days, treatment survival decreased to 80, 58, and 2 percent for the same respective exposures.

There is no significant difference in survival at equivalent concentrations of tributyltin whether introduced as the TBTO reagent or the SPC leachate after 144 hours exposure. This suggests that for purposes of toxicity testing it apparently does not matter whether the toxicant is added as the reagent or leachate. The concentration of TBTO in solution was measured during the test for both the reagent and leachate treatments (table 22). There was no decrease in concentration over time as observed in other tests because the solutions were changed daily. Actual TBTO concentrations were generally greater than the nominal values.

Test #5: February 1981

In February 1981, a 7-day test was run with juvenile mysids at TBTO concentrations 1.0, 4.0, and 22.0 ppb with three replicates of 10 animals per treatment. Control survival dropped to 96 percent after 2 days and remained there for the duration of the 7-day test. After 4 days, treatment survival was 37, 0, and 0 percent at 1.0, 4.0, and 22.0 ppb respectively. At 22.0 ppb, all the mysids died within 24 hours (table 16, figure 16).

Test #6: June 1981

In June 1981, a 10-day test was run with subadult mysids at TBTO concentrations of 1.0, 3.0, 4.0, and 11.0 ppb. There were 10 mysids per replicate and five replicates per treatment. Control survival decreased to 98 percent on day 2 and remained stable through day 4. Survival decreased again on day 7 to 94 percent and to 70 percent on day 10. In the 1.0-ppb treatment tanks, survival remained at 90 percent through day 4, dropped to 78 percent after 7 days, and 52 percent after 10 days. In the 3.0- and 4.0-ppb treatments, survival dropped rapidly to 8 and 0 percent respectively after only 4 days. The drop in survival at the highest concentration was even more dramatic. At 11.0 ppb, all the mysids died within 24 hours (table 17, figure 17).

Test #7: May 1983

In May 1983, a 4-day test was run with adult mysids and various concentrations of monobutyltin, dibutyltin, and tributyltin to determine the relative toxicity of these compounds. The concentrations tested were as follows: monobutyltin — 16.0, 161.0, and 809.0 ppb; dibutyltin — 2.0, 11.0, and 56.0 ppb; and tributyltin — 0.75, 1.5, and 6.0 ppb. Five replicates of 10 to 11 animals were used for each treatment.

Survival values are given in table 18 and figures 18a, -b, and -c. The Mann-Whitney U test ($a = 0.05$) was used to compare 4-day control survival to treatment survival. As the highest concentration of each treatment showed the lowest survival rates, data for these treatments were used for statistical analysis. There was no statistically significant difference in survival between control mysids and those exposed to 809 ppb-monobutyltin. However, statistically significant differences were obtained when the controls were compared to the 56.0-ppb dibutyltin and 6.0-ppb tributyltin treatments. After obtaining these results, statistics were also applied to data for the middle concentration of the di- and tributyltin treatments. No statistically significant differences in survival were obtained when survival for control mysids was compared to survival in either the 11.0-ppb dibutyltin or the 1.5-ppb tributyltin treatments.

ACARTIA TONSA - COPEPODS

In February and March 1981, *A tonsa* were exposed to TBTO concentrations of 1.0, 3.0, and 15.0 ppb and Cu concentrations of 10.0, 50.0, and 250.0 ppb. Treatments consisted of three replicates of 10 animals each and controls. Copepods in all of the TBTO treatment died within 24 hours. Approximately half of those in the 250-ppb Cu treatment died within 24 hours. Although control survival was low in these early copepod tests (63 percent after 96 hours), the test still gave an early indication that TBTO was at least an order of magnitude more toxic than Cu to copepods (tables 19 and 20, figures 19 and 20).

DISCUSSION

No definitive conclusions regarding the impact of organotin antifouling coatings on the marine environment can be drawn from this early work, except that bioavailable organotins are significantly more toxic than bioavailable Cu to the species we tested. The interpretation and environmental significance of organotin bioassays are difficult, even with state-of-the-art measurement techniques and bioassay procedures (Salazar, 1986). That is why these early data should be interpreted with extreme caution and used only for comparative purposes. Although absolute values presented here cannot be used to predict environmental impact, they can be used to group the test species based on relative toxicity.

Perhaps the most important aspect of this work was the lessons learned on the design and conduct of organotin toxicity tests. For example, we found that both test containers and test animals have the ability to remove organotins from test solutio[n] .nd, therefore, affect test concentrations. We also found that tributyltin can be intro[d]ced as either chemical reagents or paint leachates with similar bioassay results. Fur[the]r, we were able to confirm that for mysids organotin toxicity is roughly proportio[nal] .o the number of butyl groups, i.e., monobutyltin is less toxic than dibutyltin, whi[ch] .s less toxic than tributyltin. It also demonstrated the need for field work to valid[a]te laboratory results.

The difficulty in establishing environmental significance on 96-hour LC_{50} values is clearly demonstrated in these studies. Even if the test concentrations were relatively constant, as they were in the static renewal tests with mysids, there are many problems with 96-hour tests. First, particularly noticeable in the bivalve data, is the very high no-effect level. Studies with clams did not reveal much except that it was necessary to increase e[x]po-sure time beyond 96 hours to observe an effect. This may be attributed to insensitivity in clams, their ability to close and "physiologically shut down" for extended periods of time, and their ability to avoid toxic effects by sequestering contaminants. Further, [w]hen most of these tests were conducted in the [past], chemical measurement techniques were unsophisticated and somewhat unreliable. Even now the bioavailability and environmental significance of organotin measurements are uncertain (Salazar, 1986). These factors point to the futility of using 96-hour LC_{50} data to predict environmental impact or establish regulatory criteria, particularly with bivalves.

We could not accurately rank the relative sensitivity of the five test species to TBTO given the imprecise nature of the results. In almost every experiment where TBTO was measured, the actual concentration of TBTO in the test containers decreased by 50 percent or more during the first 4 days and as much as 90 percent or more if the test extended beyond 10 days. Relative sensitivities and LC_{50}s cannot be determined with confidence when the test concentrations are constantly changing. The species tested fell into two general groups. The first group consisted of clams, mussels, fish, and worms with approximate 4- to 14-day LC_{50}s between 20- and 30-ppb TBTO. The second group consisted of mysids and copepods with approximate 4- to 6-day LC_{50}s between 1- and 2 ppb TBTO.

Estimated LC_{50}s were used to determine the relative toxicity of organotins and Cu. Although the estimated LC_{50}s derived from this work (table B) are not precise, they show that TBTO is significantly more toxic than Cu, in most cases about 10 times more toxic. These data demonstrate that organotin AF coatings have the potential for more of an impact on the marine environment than Cu AF coatings but they cannot be used to predict the actual impact. Further, it should be remembered that all of these bioassays were performed under laboratory conditions and that natural physical, chemical, and biological processes could produce different results in the field. Therefore, even these relative toxicity comparisons should be cautiously interpreted.

Mussels exhibited greater sensitivity to TBTO than clams. The best estimate of a 10-day LC_{50} for *M. edulis*, based upon results of this study, is near 8.0-ppb TBTO. Again, this value reflects decreasing concentrations of TBTO over time. Experiments conducted during 1983 and 1984 (Valkirs, et al., 1985a) assessing the effects of chronic *TBT* exposure on mussels have indicated a 66-day LC_{50} near 1.0 ppb. These findings coincide with our earlier work in that TBT is highly toxic with LC_{50} values in the low ppb range. A 66-day LC_{50} for mussels has been estimated at 0.97-ppb TBT using probit analysis (Valkirs et al., 1985a). Even though it is difficult to compare results of different test durations, and their results were questionable due to nutritionally stressed animals and significantly fluctuating organotin concentrations, their data generally agree with our original estimate of less that 15-ppb TBTO for a 14-day LC_{50}.

Valkirs (1982) reports a 70-day LC_{40} of approximately 1.38-ppb TBT for *C. stigmaeus* maintained under flowthrough conditions. This is also in general agreement with our original estimate of a 4-day LC_{50} of less than 19-ppb TBTO.

More tests were conducted with mysids than any other animal. We feel they are the most sensitive and reliable test animal we have used. The best available data on toxicity of TBTO to adult *M. elongata* were obtained from a static-renewal test conducted in 1982. The results of the static-renewal test are the most reliable presented here because test solutions were changed daily over the first 4 days and the toxicant concentration was fairly constant. Unfortunately, the water was not changed on days 4 or 5. This was designed to be a 4-day test, but when survival remained high after 4 days, it was decided to continue the experiment. Even without water changes and without feeding on day 5, control survival remained at 90 percent on day 6. Although the latter part of the experiment can be questioned, the 6-day LC_{50} for this mysid is probably very close to 1.0-ppb TBTO. Further, it was shown conclusively for this particular species that it makes no difference whether the toxicant is added as TBTO chemical or paint leachate. The static-renewal test also provided the best available estimate of acute toxicity of TBTO to adult mysids.

We believe that mysids are the most appropriate and sensitive test animal we have used for organotin bioassays, even though in the early organotin tests with *M. elongata* we experienced some variability. This variability was attributed to using field-caught animals, improving animal handling and maintenance techniques, and more sensitive and repeatable chemical measurements. Although later work would confirm that our original 96-hour LC_{50} estimate of 2-ppb TBTO was reasonable for mysids, a true comparison cannot be made for two reasons. First, we used *M. elongata* in toxicity studies until 1983, when we selected *Acanthomysis sculpta* for such studies. *A. sculpta* was used because it was easier to collect, maintain, and count. In addition, it could be used with the flowthrough bioassay system developed in 1982. Second, chemical measurement techniques continued to improve resulting in more reliable organotin determinations. Therefore, we cannot make direct comparisons between these different test conditions. These data should only be used as a guide.

The copepod, *A. tonsa*, was the most sensitive animal we tested; however, it was also the most variable. As mentioned previously, the copepod data are the least reliable of all because there is significant natural variability in field-caught animals and our maintenance techniques were still being developed. Even with low control survival, the first test conducted in 1981 showed that copepods were very sensitive to TBTO and that the 96-hour LC_{50} was probably less than 1.0 ppb. This is in agreement with the 96-hr LC_{50} of 1.0 ppb reported by U'Ren (1983). For comparative purposes, the 96-hour LC_{50} for Cu was near 10.0 ppb.

The loss of organotin from solution due to adsorption was a severe problem throughout these studies. Our research on the adsorptive properties of various materials indicated polycarbonate and high quality glass, such as Pyrex, were relatively nonadsorptive (Dooley and Homer, 1983), while the standard glass aquaria used in the first tests with clams and mussels were highly adsorptive. Chemical analyses made on seawater from the clam and mussel tests collected at 24 and 96 hours indicated losses up to 50 and 90 percent respectively. We replaced all test aquaria with one-piece molded polycarbonate tanks for subsequent organotin toxicity tests. Pyrex beakers were used for copepods because they required smaller volumes of test solution. In addition to glassware adsorbing the toxicant, the animals themselves have the capability of decreasing available organotin. Results indicate that mussels were responsible for uptake of nearly 45 percent of the available organotin. However, it is not known what portion of this 45 percent was adsorbed on the shell material or what portion was bioaccumulated in the tissues of the animals. It is clear, however, that there was a significant loss of TBTO from solution due to uptake by animals and the glass tanks.

One of the most important findings of the early toxicity tests on the acute effects of (bis)tributyltin oxide on marine organisms was that organotins were about an order of magnitude more toxic than Cu to the species tested. Our initial reaction was that this compound was so toxic additional work would only confirm that TBTO should not be used as the active ingredient in antifouling paints. As the work progressed, however, that conclusion became less certain. We could not accurately rank the sensitivity of test species but only group them into resistant and sensitive categories. In the first group with 96-hour LC_{50s} roughly between 15- and 30-ppb TBTO are *Protothaca staminea, Mytilus edulis, Citharichthys Stigmaeus,* and *Neanthes arenaceodentata.* In the second group with 96-hour LC_{50s} roughly between 1- and 2-ppb TBTO are *Metamysidopsis elongata* and *Acartia tonsa.*

Other important results included significant findings on the design and conduct of laboratory toxicity tests with TBTO. Mysid tests demonstrated that tributyltin can be introduced as either the chemical reagent or leachate with similar results. This finding was crucial to our decision to switch to leachate testing for future flowthrough experiments with mysids (Salazar and Salazar, 1985; Davidson et al., 1986; Valkirs et al., 1987), PETS experiments (Salazar et al., 1987; Henderson, 1986), and our current field-dosing experiment. It provided a much cheaper and easier method of introducing TBTO toxicants to test systems. Equally important was the finding that both test containers and test animals can uptake organotins from solution. Further, the quality and type of test container materials greatly influences adsorption. Based on mysid tests, organotin toxicity appears roughly proportional to the number of butyl groups present, i.e., toxicity can be ranked as follows: monobutyltin $<$ dibutyltin $<$tributyltin.

Our most significant finding was that these results did not appear meaningful in an environmental perspective. The authors' frustration with lack of meaningful results led to development of new laboratory techniques for TBTO testing that included static renewal and flowthrough approaches with and without sediment. It also encouraged the development of flowthrough microcosm testing and field-dosing experiments using TBTO leachates. In addition, these early acute studies led us from the laboratory to the field where we have studied the effects of TBTO on mussel growth under natural conditions. Field assessment and validation studies are essential to environmental prediction of TBTO effects, as current field studies suggest laboratory studies alone may not accurately predict environmental impacts.

SUMMARY AND CONCLUSIONS

1. Organotins were significantly more toxic than Cu to all of the species we tested by about a factor of 10.

2. We were not able to rank the sensitivity of test species with confidence but were able to group them into resistant and sensitive categories. In the first group with 96-hour LC_{50}s roughly between 15- and 30-ppb TBTO are *Protothaca staminea, Mytilus edulis, Citharichthys stigmaeus,* and *Neanthes arenaceodentata.* In the second group with 96-hour LC_{50}s roughly between 1- and 2-ppb TBTO are *Metamysidopsis elongata* and *Acartia tonsa.*

3. In toxicity tests, tributyltin can be introduced as either the chemical reagent or as a component of the paint leachate and still yield similar effects.

4. Both the test container and animals can uptake organotins from solution. The qualand type of test container materials greatly influences adsorption.

5. Based on mysid tests, organotin toxicity appears roughly proportional to the number of butyl groups present, i.e., monobutyltin is less toxic than dibutyltin which is less toxic than tributyltin.

Table 1. Survival of *Mytilus edulis* (mussels) exposed to TBTO (14 January 1981). TBTO concentrations were measured by GF-AAS analysis.

		Time (Days)					
Treatment	Replicate	0	2	3	4	7	10
Control	1	10	10	10	10	10	10
	2	10	10	10	10	10	10
	3	10	10	10	10	10	109
		100%	100%	100%	100%	100%	100%
3.0 ppb	1	10	10	10	10	10	10
	2	10	10	10	10	10	10
	3	10	10	10	10	10	10
		100%	100%	100%	100%	100%	100%
15.0 ppb	1	10	10	8	8	6	3
	2	10	10	9	9	6	6
	3	10	9	8	7	6	6
		100%	97%	83%	80%	60%	50%
76.0 ppb	1	10	5	3	1	0	0
	2	10	9	3	1	0	0
	3	10	10	4	1	0	0
		100%	80%	33%	10%	0%	0%

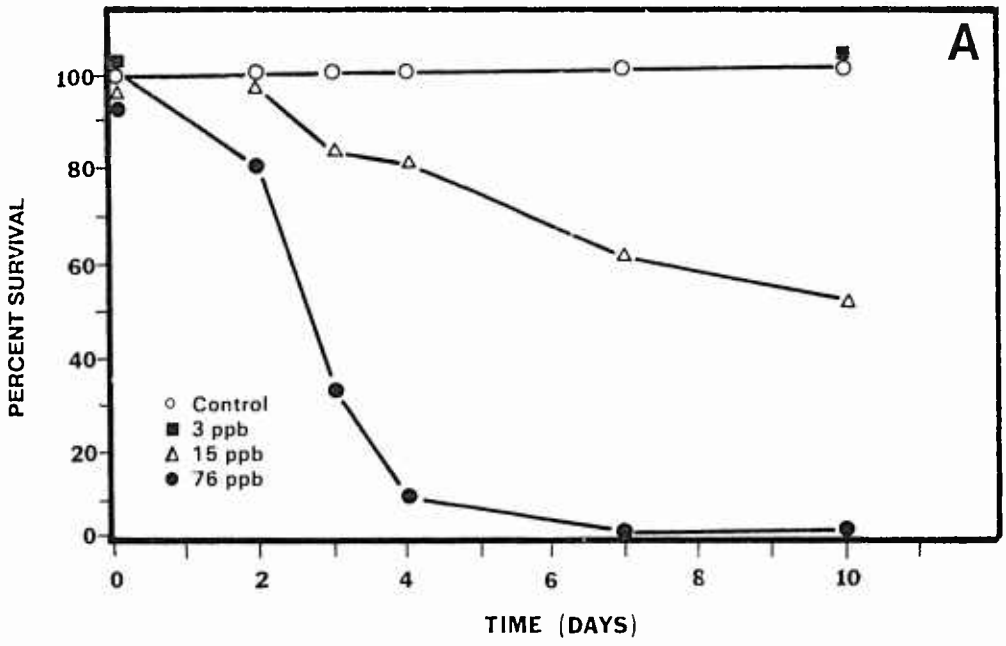

Figure 1. Survival of mussels exposed to TBTO for 10 days in January 1981.

Table 2. Survival of *Protothaca staminea* (clams) exposed to TBTO (14 January 1981). TBTO concentrations were measured by GF-AAS analysis.

		Time (Days)					
Treatment	Replicate	0	2	3	4	7	10
Control	1	10	10	10	10	10	10
	2	10	10	10	10	10	10
	3	10	10	10	10	10	10
		100%	100%	100%	100%	100%	100%
3.0 ppb	1	10	10	10	10	10	10
	2	10	10	10	10	10	9
	3	10	10	10	10	10	10
		100%	100%	100%	100%	100%	97%
15.0 ppb	1	10	10	10	10	10	10
	2	10	10	10	10	10	10
	3	10	10	10	10	10	10
		100%	100%	100%	100%	100%	100%
76.0 ppb	1	10	10	10	10	9	6
	2	10	10	10	10	10	8
	3	10	10	10	10	10	10
		100%	100%	100%	100%	97%	80%

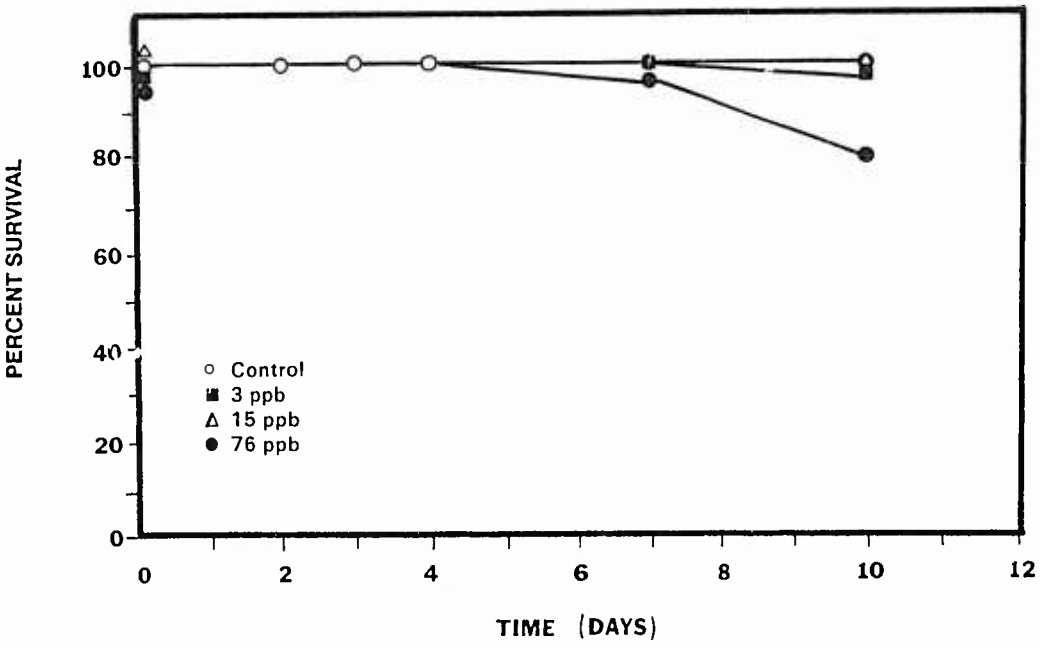

Figure 2. Survival of clams exposed to TBTO for 10 days in January 1981.

Table 3. Survival of *Protothaca staminea* (clams) exposed to TBTO (7 April 1981). TBTO concentrations were measured by GF-AAS analysis.

		Time (Days)								
Treatment	Replicate	0	1	2	3	4	6	8	9	13
Control	1	10	10	10	10	10	10	10	10	10
	2	10	10	10	10	10	10	10	10	10
	3	10	10	10	10	10	9	9	9	9
		100%	100%	100%	100%	100%	97%	97%	97%	97%
40.0 ppb	1	10	10	10	10	10	10	10	10	10
	2	10	10	10	10	10	10	10	10	10
	3	10	10	10	10	10	8	7	6	3
		100%	100%	100%	100%	100%	93%	90%	87%	77%
119.0 ppb	1	10	10	10	10	10	8	5	5	2
	2	10	10	10	10	10	10	7	7	0
	3	10	10	10	10	10	9	7	6	5
		100%	100%	100%	100%	100%	90%	63%	60%	23%
271.0 ppb	1	10	10	10	10	10	9	8	7	2
	2	10	10	10	10	10	9	6	6	1
	3	10	10	10	10	10	9	7	7	5
		100%	100%	100%	100%	100%	90%	70%	67%	27%

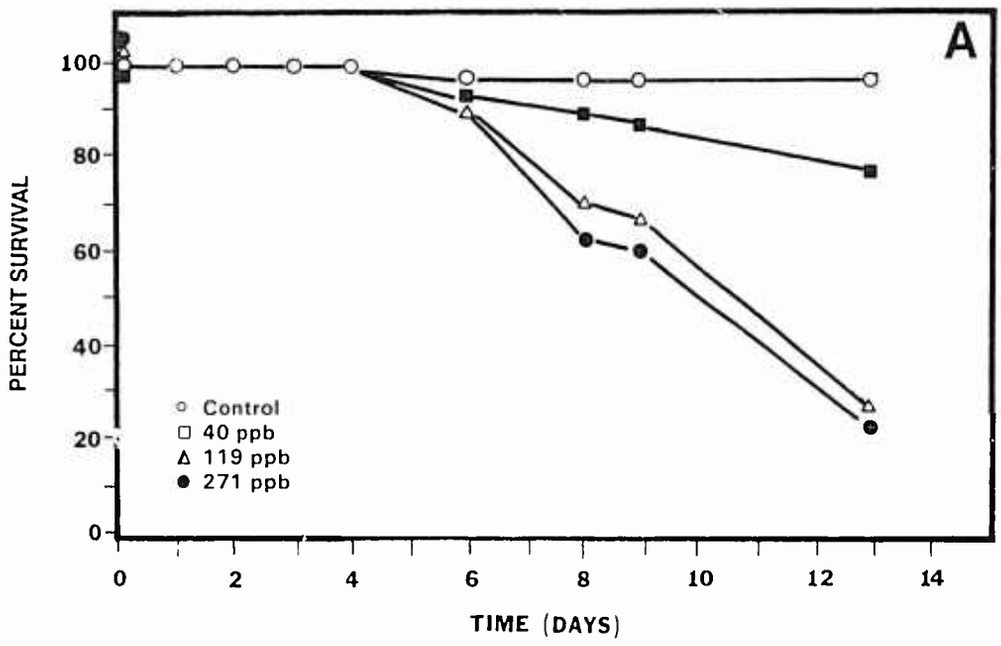

Figure 3. Survival of clams exposed to TBTO for 13 days in April 1981.

Table 4. Survival of *Protothaca staminea* (clams) exposed to copper (6 April 1981). Copper concentrations were theoretical.

		Time (Days)								
Treatment	Replicate	0	1	2	3	5	7	9	10	14
Control	1	10	10	10	10	10	10	10	10	10
	2	10	10	10	10	10	10	10	10	10
	3	10	10	10	10	10	9	9	9	9
		100%	100%	100%	100%	100%	97%	97%	97%	97%
250 ppb	1	10	10	10	10	10	8	8	7	5
	2	10	10	10	10	10	9	8	7	6
	3	10	10	10	10	10	9	9	9	5
		100%	100%	100%	100%	100%	87%	83%	77%	53%
750 ppb	1	10	10	10	10	9	5	5	5	4
	2	10	10	9	9	9	8	7	6	2
	3	10	10	9	9	9	7	7	6	6
		100%	100%	97%	97%	90%	67%	63%	57%	40%
2250 ppb	1	10	10	10	9	9	5	5	5	5
	2	10	10	10	10	10	6	5	5	1
	3	10	10	10	10	10	9	9	8	2
		100%	100%	100%	97%	97%	67%	63%	60%	27%

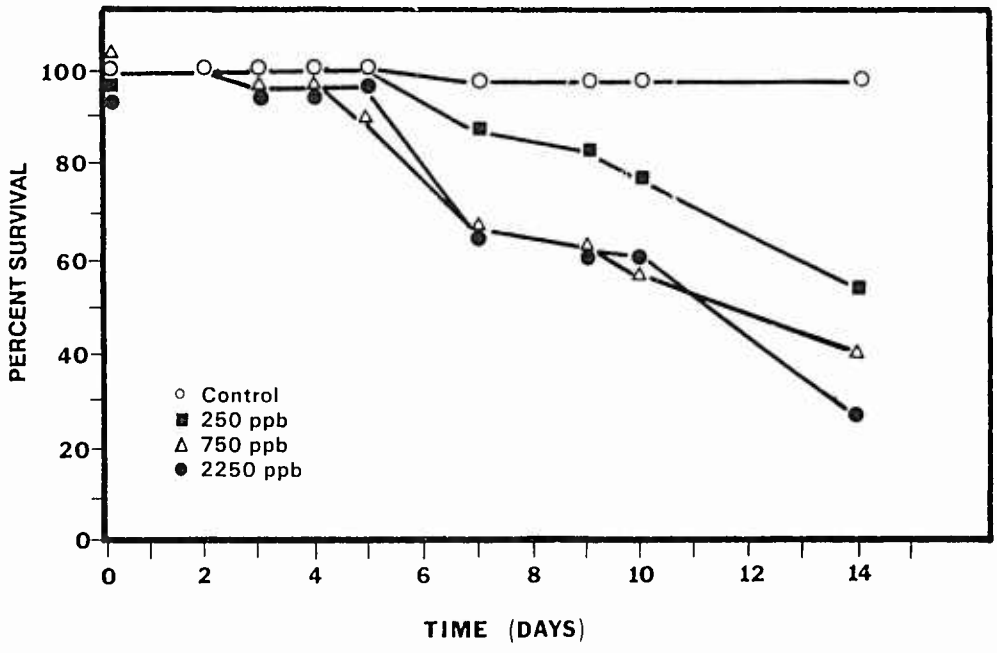

Figure 4. Survival of clams exposed to copper for 14 days in April 1981.

Table 5. Survival of *Citharichthys stigmaeus* (fish) exposed to TBTO (6 April 1981). TBTO concentrations were measured by GF-AAS analysis.

		Time (Days)									
Treatment	Replicate	0	1	2	3	4	5	7	9	10	14
Control	1	10	9	9	9	6	6	6	6	6	6
	2	10	10	10	10	10	10	10	10	10	10
	3	10	9	9	9	8	8	7	7	7	5
		100%	97%	97%	97%	80%	80%	77%	77%	77%	70%
3.0 ppb	1	10	10	10	9	9	9	9	9	9	5
	2	10	10	8	8	8	8	8	8	8	8
	3	10	10	9	9	9	8	7	7	7	5
		100%	100%	90%	87%	87%	83%	80%	80%	80%	60%
19.0 ppb	1	10	10	8	7	7	7	6	6	6	5
	2	10	10	7	4	2	2	2	2	2	2
	3	10	10	6	6	6	6	6	6	6	6
		100%	100%	70%	57%	50%	50%	47%	47%	47%	43%
123.0 ppb	1	10	10	0	0	0	0	0	0	0	0
	2	10	10	0	0	0	0	0	0	0	0
	3	10	10	0	0	0	0	0	0	0	0
		100%	100%	0%	0%	0%	0%	0%	0%	0%	0%

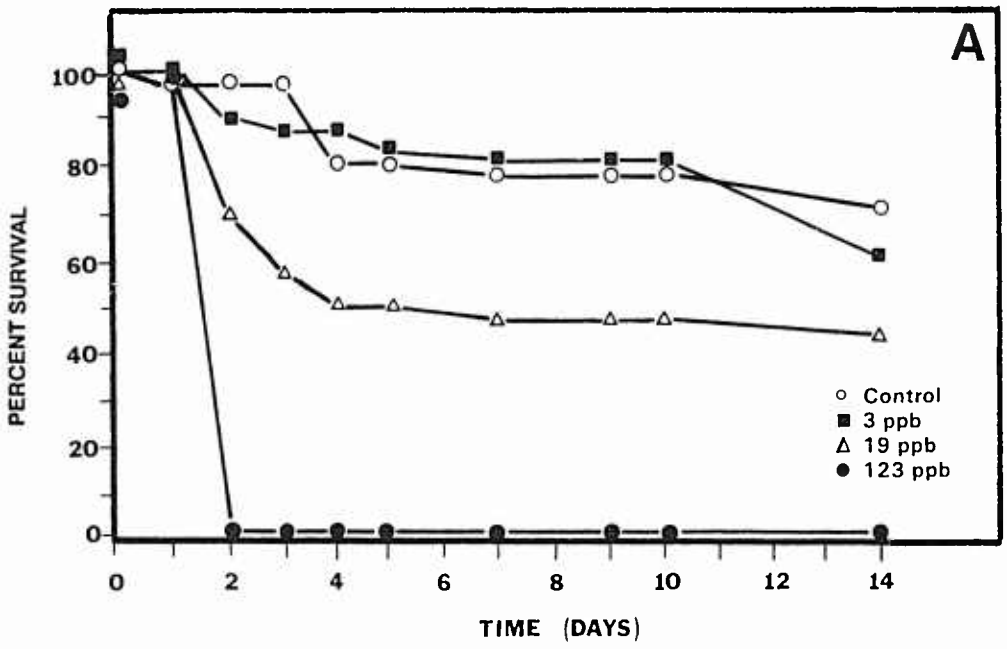

Figure 5. Survival of fish exposed to TBTO for 14 days in April 1981.

Table 6. Survival of *Citharichthys stigmaeus* (fish) exposed to copper (6 April 1981). Copper concentrations were theoretical.

		Time (Days)									
Treatment	Replicate	0	1	2	3	4	5	7	9	10	14
Control	1	10	9	9	9	6	6	6	6	6	6
	2	10	10	10	10	10	10	10	10	10	10
	3	10	9	9	9	8	8	7	7	7	5
		100%	97%	97%	97%	80%	80%	77%	77%	77%	70%
250.0 ppb	1	10	7	7	7	6	5	4	3	2	1
	2	10	4	3	3	3	3	3	3	3	2
	3	10	9	7	5	5	5	4	4	4	4
		100%	67%	57%	50%	47%	43%	37%	33%	30%	23%
750.0 ppb	1	10	6	5	5	4	4	4	2	1	0
	2	10	8	8	8	6	6	6	5	1	0
	3	10	8	8	8	6	6	4	2	1	0
		100%	70%	67%	67%	60%	60%	47%	30%	10%	0%
2250.0 ppb	1	10	7	7	7	2	0	0	0	0	0
	2	10	10	10	6	1	0	0	0	0	0
	3	10	10	8	7	1	0	0	0	0	0
		100%	90%	83%	67%	13%	0%	0%	0%	0%	0%

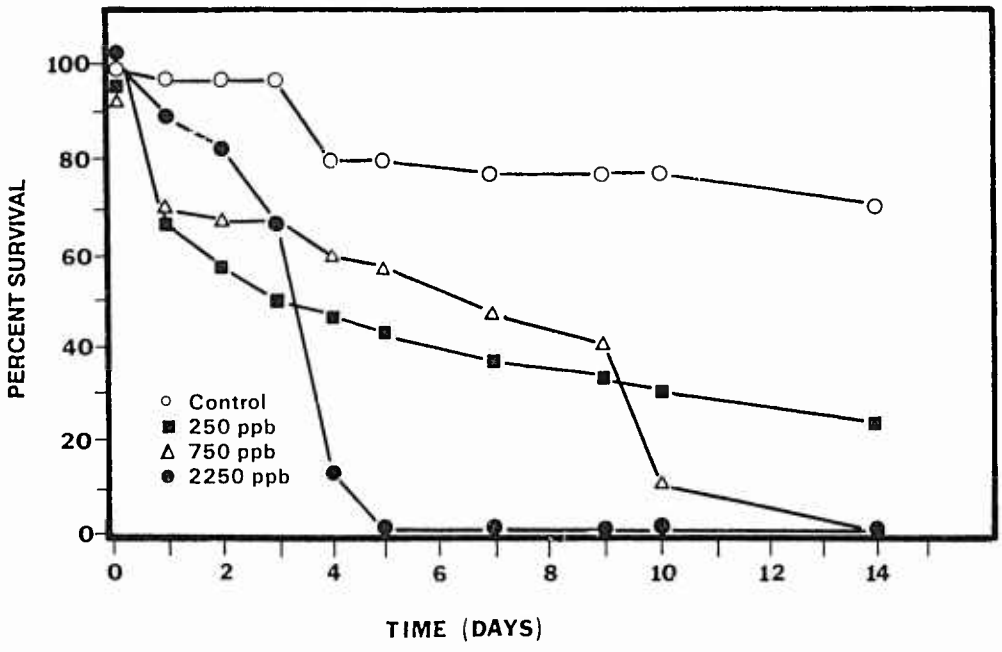

Figure 6. Survival of fish exposed to copper for 14 days in April 1981.

Table 7. Survival of adult *Neanthes arenaceodentata* (worms) exposed to TBTO (7 April 1981). TBTO concentrations were measured by GF-AAS analysis.

		Time (Days)					
Treatment	Replicate	0	1	2	3	4	6
Control	1	10	10	10	10	10	10
	2	10	10	10	10	9	9
	3	10	10	10	10	10	10
		100%	100%	100%	100%	97%	97%
10.0 ppb	1	10	10	10	10	10	10
(Nominal)	2	10	10	9	9	9	9
	3	10	10	10	10	10	9
		100%	100%	97%	97%	97%	93%
35.0 ppb	1	10	10	5	0	0	0
	2	10	10	3	0	0	0
	3	10	9	0	0	0	0
		100%	97%	27%	0%	0%	0%
150.0 ppb	1	10	0	0	0	0	0
	2	10	0	0	0	0	0
	3	10	0	0	0	0	0
		100%	0%	0%	0%	0%	0%

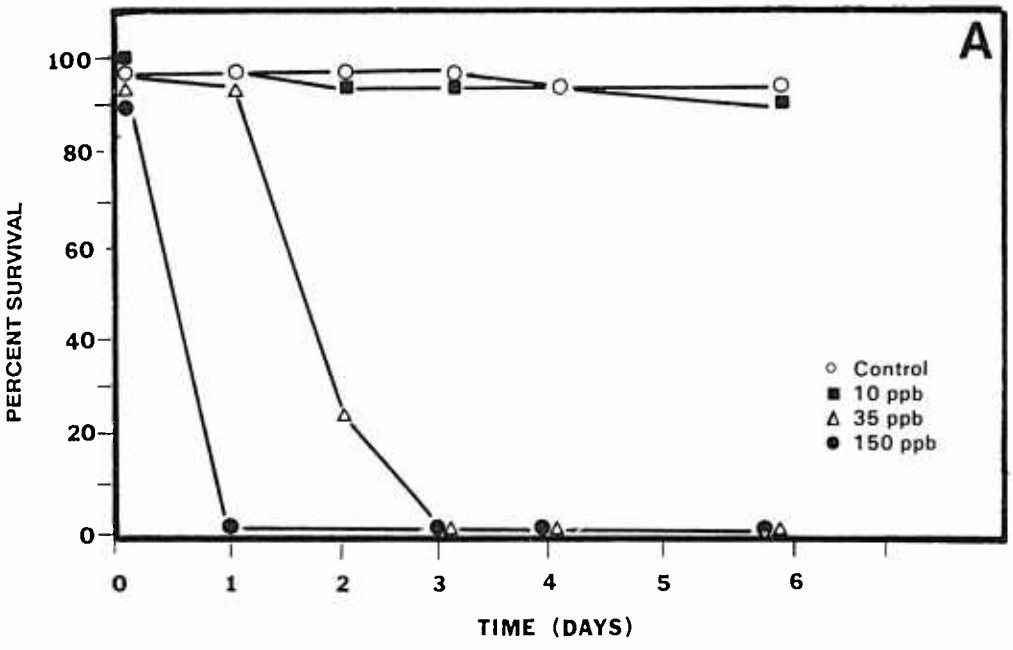

Figure 7. Survival of worms exposed to TBTO for 6 days in April 1981.

Table 8. Survival of adult *Neanthes arenaceodentata* (worms) exposed to copper (7 January 1981). Copper concentrations were theoretical.

		Time (Days)					
		0	1	2	3	4	6
Treatment	Replicate						
Control	1	10	10	10	10	10	10
	2	10	10	10	10	9	9
	3	10	10	10	10	10	10
		100%	100%	100%	100%	97%	97%
250.0 ppb	1	10	10	10	9	8	0
	2	10	10	10	9	6	0
	3	10	10	10	8	4	0
		100%	100%	100%	87%	60%	0%
750.0 ppb	1	10	10	0	0	0	0
	2	10	10	4	0	0	0
	3	10	10	0	0	0	0
		100%	100%	13%	0%	0%	0%
2250.0 ppb	1	10	0	0	0	0	0
	2	10	0	0	0	0	0
	3	10	1	0	0	0	0
		100%	3%	0%	0%	0%	0%

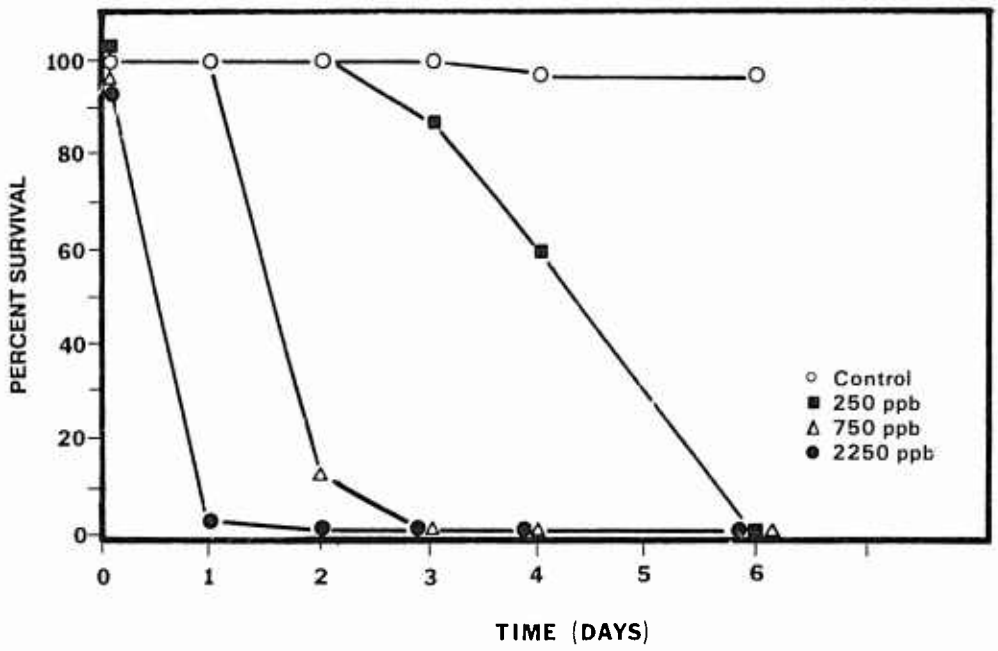

Figure 8. Survival of worms exposed to copper for 6 days in April 1981.

Table 9. Survival of juvenile *Neanthes arenaceodentata* (worms) exposed to TBTO (27 July 1981). TBTO concentrations were measured by GF-AAS analysis. Number of worms per treatment is given in parentheses (n).

		Time (Days)				
		0	1	2	3	4
Treatment	Replicate					
Control	1	10	10	10	10	10
	2	10	10	10	10	10
	3	10	10	10	10	10
	4	10	10	10	10	10
	5	10	10	10	10	10
		100%	100%	100%	100%	100%
4.0 ppb	1	11	11	11	10	7
(52)	2	10	10	10	10	10
	3	10	10	10	10	9
	4	10	9	9	9	8
	5	11	11	11	11	10
		100%	98%	98%	96%	85%
10.0 ppb	1	10	10	9	5	0
(51)	2	11	11	4	0	0
	3	10	10	6	2	0
	4	10	10	0	1	0
	5	10	9	5	0	0
		100%	94%	57%	16%	0%
14.0 ppb	1	10	10	0	0	0
(50)	2	10	9	0	0	0
	3	10	10	2	1	0
	4	10	10	3	0	0
	5	10	9	0	0	0
		100%	98%	10%	2%	0%
20.0 ppb	1	10	8	0	0	0
(50)	2	10	9	0	0	0
	3	10	7	0	0	0
	4	10	9	0	0	0
	5	10	9	0	0	0
		100%	84%	0%	0%	0%

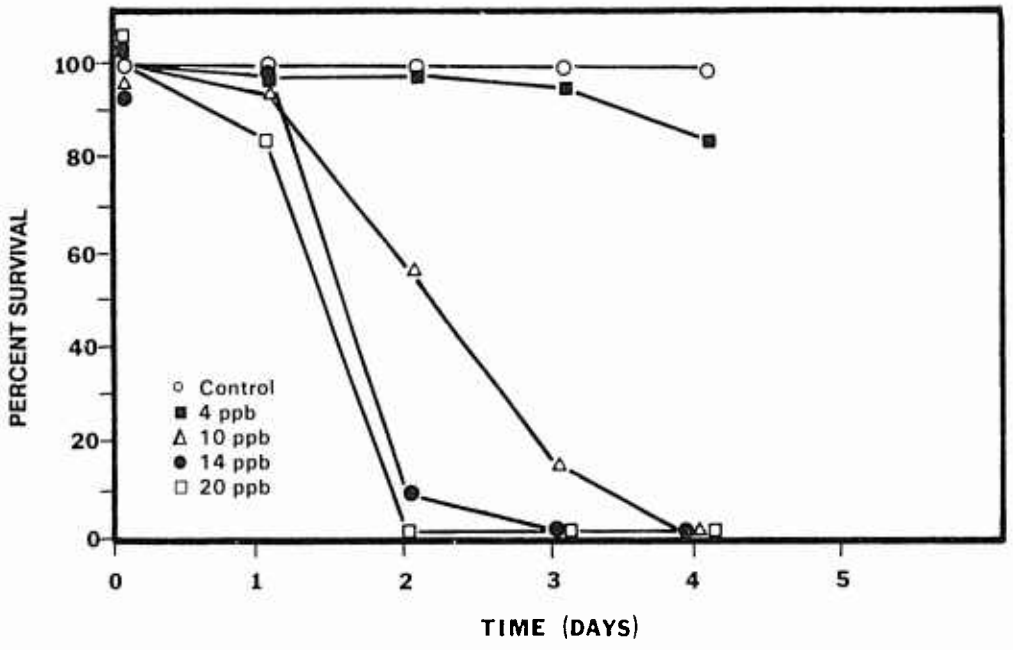

Figure 9. Survival of juvenile worms exposed to TBTO for 4 days in July 1981.

Table 10. Survival of adult *Metamysidopsis elongata* (mysids) exposed to TBTO (12 January 1981). TBTO concentrations were measured by GF-AAS analysis.

		Time (Days)					
		0	1	2	3	4	7
Treatment	Replicate						
Control	1	10	10	10	10	10	10
	2	10	10	10	10	10	10
	3	10	10	9	9	9	9
		100%	100%	97%	97%	97%	97%
1.0 ppb	1	10	10	9	9	9	9
	2	10	10	10	10	10	10
	3	10	10	10	10	10	9
		100%	100%	97%	97%	97%	93%
3.0 ppb	1	10	10	10	10	7	0
	2	10	10	10	10	9	4
	3	10	10	10	10	9	6
		100%	100%	100%	100%	83%	33%
15.0 ppb	1	10	8	3	1	0	0
	2	10	6	1	0	0	0
	3	10	6	2	0	0	0
		100%	67%	20%	3%	0%	0%

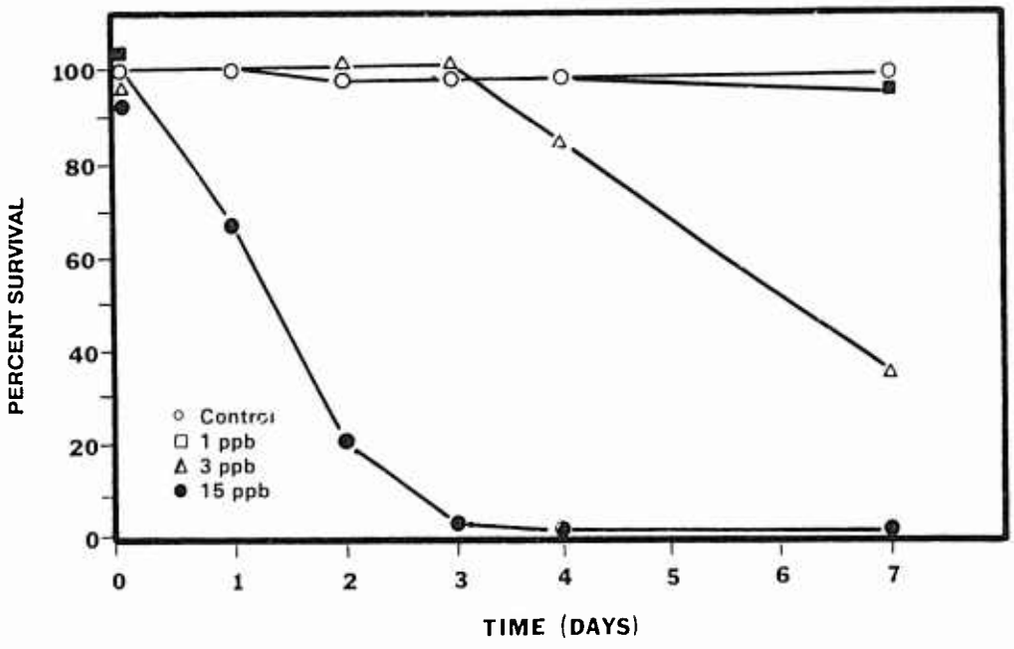

Figure 10. Survival of mysids exposed to TBTO for 7 days in January 1981.

Table 11. Survival of adult *Metamysidopsis elongata* (mydids) exposed to TBTO (7 April 1981). TBTO concentrations were nominal.

		Time (Days)					
Treatment	Replicate	0	1	2	3	4	5
Control	1	10	10	9	9	8	3
	2	10	10	10	9	8	7
	3	10	10	9	7	7	4
		100%	100%	93%	83%	77%	47%
2.0 ppb	1	10	10	7	3	0	0
	2	10	10	8	2	1	1
	3	10	10	6	4	3	0
		100%	100%	70%	30%	17%	3%
10.0 ppb	1	10	7	1	0	0	0
	2	10	9	1	0	0	0
	3	10	7	2	0	0	0
		100%	77%	13%	0%	0%	0%
14.0 ppb	1	10	1	0	0	0	0
	2	10	2	0	0	0	0
	3	10	2	0	0	0	0
		100%	17%	0%	0%	0%	0%

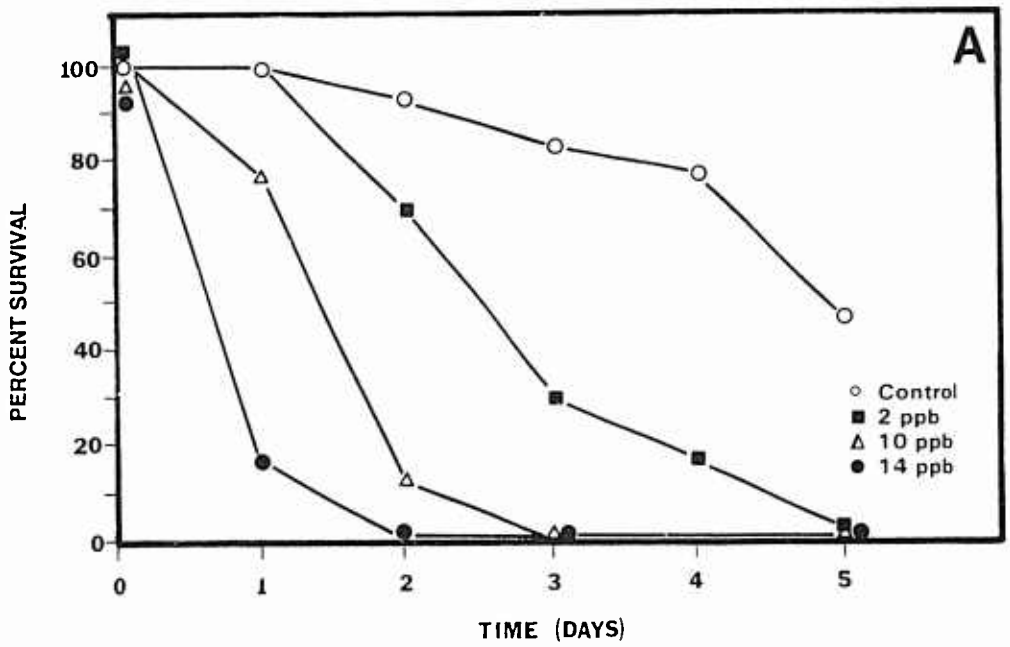

Figure 11. Survival of mysids exposed to TBTO for 5 days in April 1981.

Table 12. Survival of adult *Metamysidopsis elongata* (mysids) exposed to copper (7 April 1981). Copper concentrations were theoretical.

		Time (Days)						
		0	1	2	3	4	5	7
Treatment	Replicate							
Control	1	10	10	9	9	8	3	1
	2	10	10	10	9	8	7	0
	3	10	10	9	7	7	4	1
		100%	100%	93%	83%	77%	47%	7%
10.0 ppb	1	10	10	9	8	8	4	0
	2	10	10	8	8	8	6	0
	3	10	10	9	7	7	2	0
		100%	100%	87%	77%	77%	40%	0%
30.0 ppb	1	10	10	8	4	2	2	2
	2	10	10	8	2	0	0	1
	3	10	9	8	2	2	2	0
		100%	97%	80%	27%	13%	13%	10%
90.0 ppb	1	10	7	1	0	0	0	0
	2	10	6	5	2	0	0	0
	3	10	5	3	1	1	0	0
		100%	60%	30%	10%	3%	0%	0%

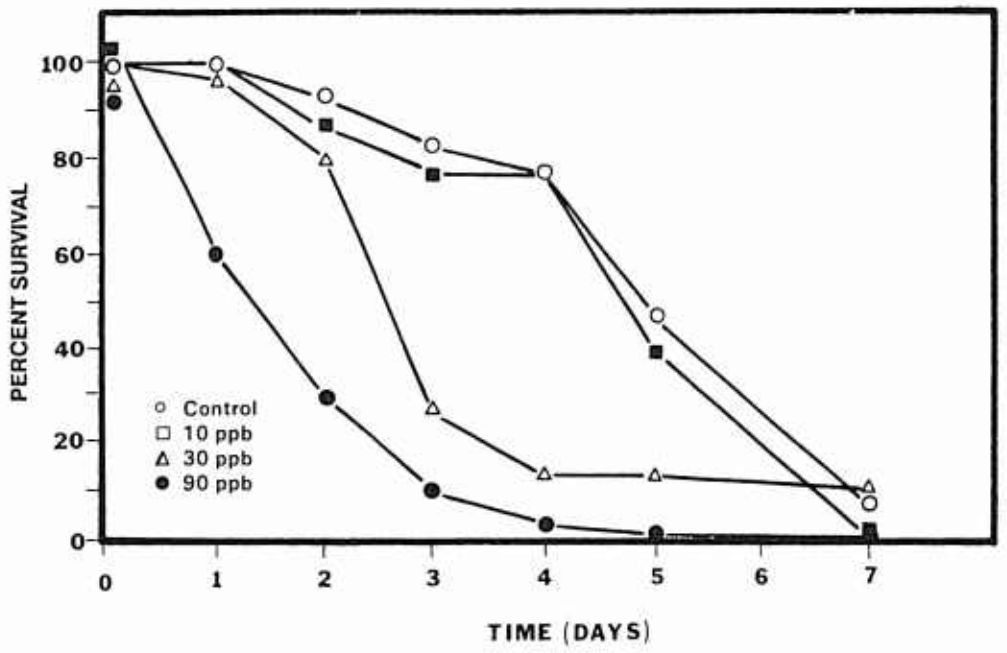

Figure 12. Survival of mysids exposed to copper for 7 days in April 1981.

Table 13. Survival of adult *Metamysidopsis elongata* (mysids) exposed to TBTO (27 July 1981). TBTO concentrations were measured by GF-AAS analysis. Number of mysids per treatment is given in parentheses (n).

		Time (Days)				
Treatment	Replicate	0	1	2	3	4
Control	1	9	9	9	8	8
(56)	2	12	12	12	12	9
	3	12	12	12	12	11
	4	11	10	10	10	10
	5	12	12	11	11	10
		100%	98%	96%	95%	86%
<0.2 ppb	1	10	8	8	7	4
(54)	2	11	9	9	9	6
	3	10	9	9	8	6
	4	11	10	8	6	4
	5	12	11	11	8	8
		100%	87%	83%	70%	52%
1.0 ppb	1	10	9	7	3	0
(52)	2	11	10	8	4	1
	3	10	10	8	3	2
	4	10	10	8	3	2
	5	11	10	7	2	1
		100%	94%	73%	29%	12%
3.0 ppb	1	9	7	2	1	0
(50)	2	10	8	5	0	0
	3	10	7	4	1	1
	4	10	9	3	0	0
	5	11	0	0	0	0
		100%	62%	28%	4%	2%
8.0 ppb	1	9	0	0	0	0
(50)	2	10	2	0	0	0
	3	10	1	0	0	0
	4	10	0	0	0	0
	5	11	1	0	0	0
		100%	8%	0%	0%	0%

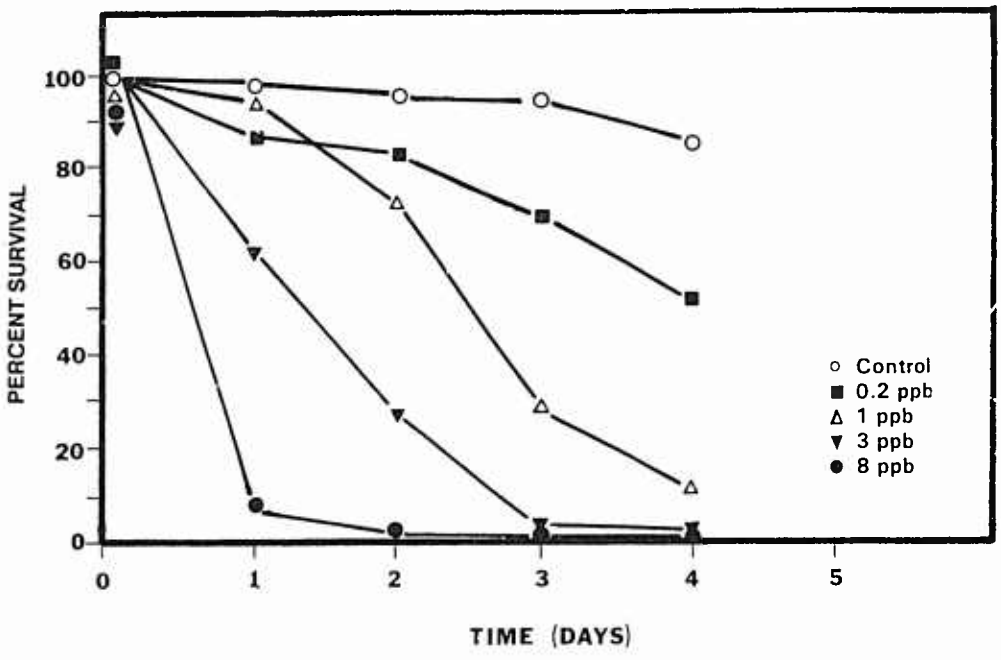

Figure 13. Survival of mysids exposed to TBTO for 4 days in July 1981.

Table 14. Survival of adult *Metamysidopsis elongata* (mysids) exposed to TBTO under static-renewal conditions (16 November 1981). TBTO concentrations were nominal.

		Time (Days)					
		0	1	2	3	4	6
Treatment	Replicate						
Control	1	10	10	9	8	8	8
	2	10	10	10	10	10	10
	3	10	9	9	9	9	9
	4	10	10	10	10	10	10
	5	10	9	9	8	8	8
		100%	96%	94%	90%	90%	90%
0.25 ppb	1	10	10	10	10	10	10
	2	10	8	6	6	6	5
	3	10	10	9	9	8	8
	4	10	10	9	9	9	9
	5	10	10	10	10	10	8
		100%	96%	88%	88%	86%	80%
1.0 ppb	1	10	9	9	9	8	3
	2	10	10	10	10	10	9
	3	10	9	8	8	8	8
	4	10	10	10	10	7	2
	5	10	10	9	7	7	6
		100%	96%	92%	88%	80%	56%
4.0 ppb	1	10	8	6	4	1	0
	2	10	10	8	6	2	1
	3	10	8	6	3	2	0
	4	10	9	4	2	0	0
	5	10	9	6	4	3	0
		100%	88%	60%	38%	16%	2%

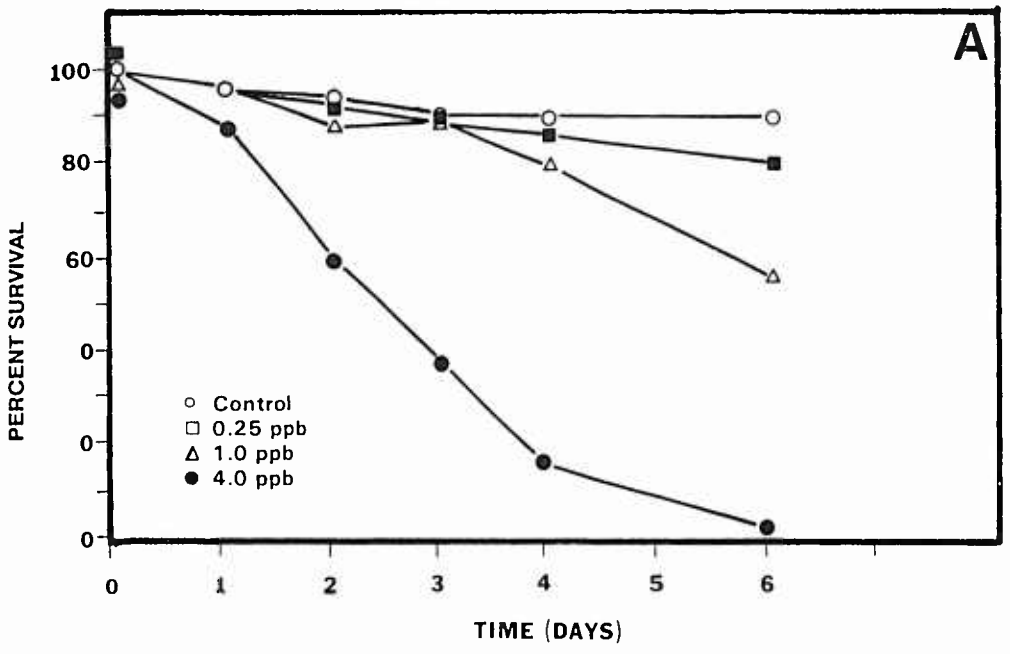

Figure 14. Survival of mysids exposed to TBTO under static-renewal conditions for 6 days in November 1982.

Table 15. Survival of adult *Metamysidopsis elongata* (mysids) exposed to SPC-leachate under static-renewal conditions (16 November 1982). SPC-leachate concentrations were nominal.

		Time (Days)					
		0	1	2	3	4	6
Treatment	Replicate						
Control	1	10	10	9	8	8	8
	2	10	10	10	10	10	10
	3	10	10	10	10	10	10
	4	10	9	9	9	9	9
	5	10	9	9	8	8	8
		100%	96%	94%	90%	90%	90%
0.25 ppb	1	10	9	9	8	8	5
	2	10	10	9	9	9	9
	3	10	9	9	8	8	8
	4	10	9	9	9	9	9
	5	10	10	9	9	9	9
		100%	94%	90%	86%	86%	80%
1.0 ppb	1	10	9	9	9	9	8
	2	10	10	9	9	6	1
	3	10	10	10	10	10	9
	4	10	10	10	10	10	9
	5	10	9	8	8	6	2
		100%	96%	92%	92%	82%	58%
4.0 ppb	1	10	8	6	4	3	0
	2	10	10	7	5	3	0
	3	10	9	8	3	2	0
	4	10	10	9	4	0	0
	5	10	9	9	5	2	1
		100%	94%	83%	42%	20%	2%

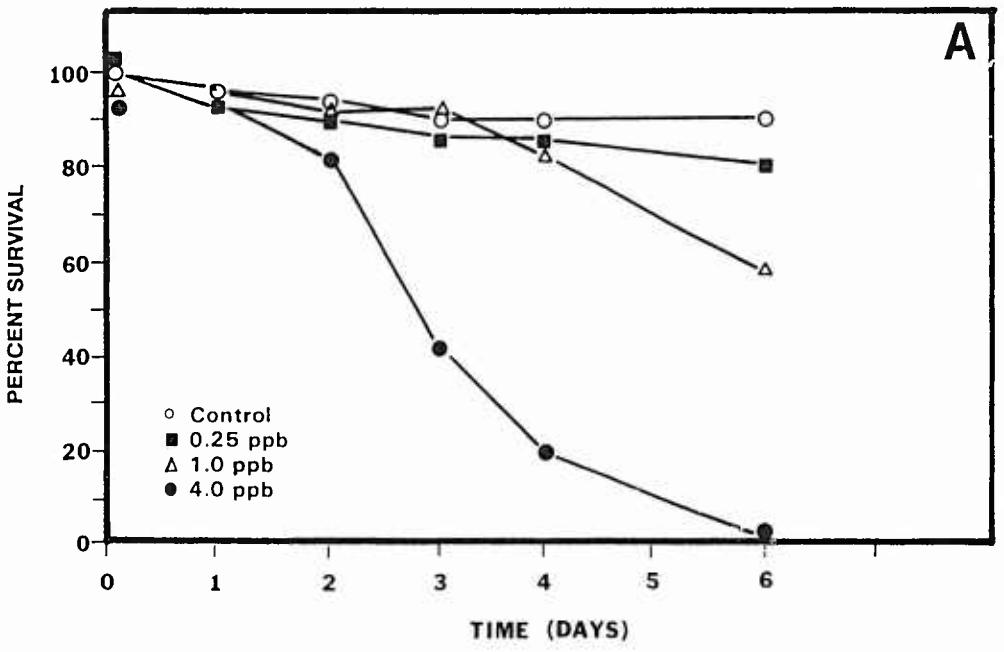

Figure 15. Survival of mysids exposed to SPC-leachate for 6 days in November 1982.

Table 16. Survival of juvenile *Metamysidopsis elongata* (mysids) exposed to TBTO (10 February 1981). TBTO concentrations were measured by GF-AAS analysis.

		Time (Days)					
		0	1	2	3	4	7
Treatment	Replicate						
Control	1	9	9	8	8	8	8
	2	9	9	9	9	9	9
	3	9	9	9	9	9	9
		100%	100%	96%	96%	96%	96%
1.0 ppb	1	9	9	9	6	2	2
	2	9	9	9	5	4	1
	3	9	9	8	5	4	3
		100%	100%	96%	59%	37%	22%
4.0 ppb	1	9	5	4	0	0	0
	2	9	4	1	0	0	0
	3	9	5	3	0	0	0
		100%	52%	30%	0%	0%	0%
22.0 ppb	1	9	0	0	0	0	0
	2	9	0	0	0	0	0
	3	9	0	0	0	0	0
		100%	0%	0%	0%	0%	0%

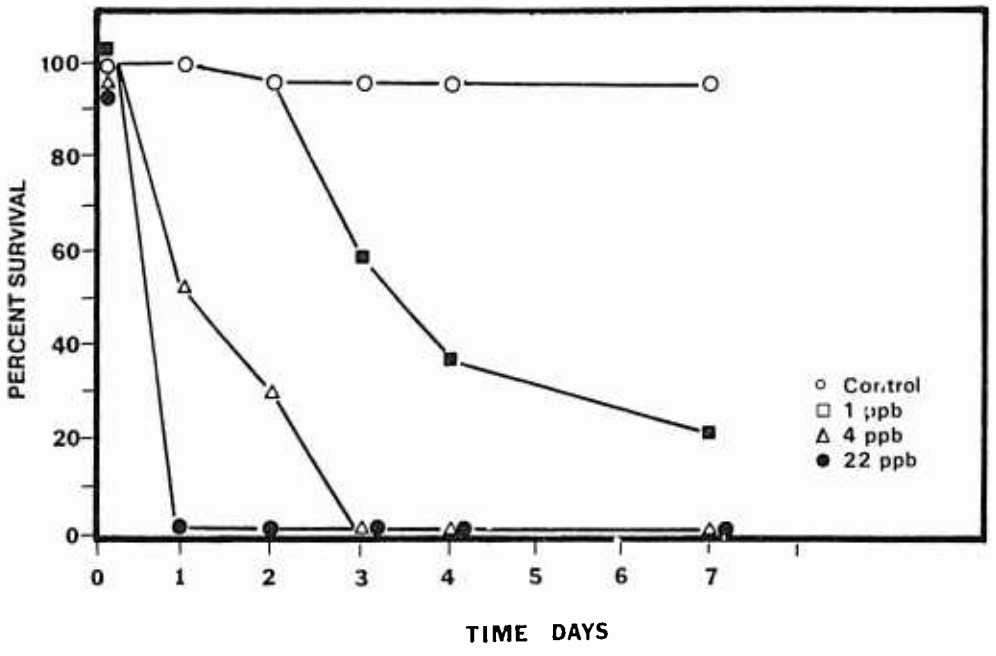

Figure 16. Survival of juvenile mysids exposed to TBTO for 7 days in February 1981.

Table 17. Survival of subadult *Metamysidopsis elongata* (worms) exposed to TBTO (1 June 1981). TBTO concentrations were measured by GF-AAS analysis.

		Time (Days)								
Treatment	Replicate	0	1	2	3	4	7	8	9	10
Control	1	10	10	10	10	10	10	8	7	6
	2	10	10	10	10	10	10	7	7	7
	3	10	10	10	10	10	10	9	8	8
	4	26	10	9	9	9	7	7	7	6
	5	10	10	10	10	10	10	10	9	8
		100%	100%	98%	98%	98%	94%	82%	76%	70%
1.0 ppb	1	10	9	9	8	7	7	7	7	7
	2	10	10	10	10	9	6	6	6	6
	3	10	10	10	10	10	8	8	8	8
	4	10	10	10	9	9	8	5	2	1
	5	10	10	10	10	10	10	5	4	4
		100%	98%	98%	94%	90%	78%	62%	54%	52%
3.0 ppb	1	10	10	8	1	1	0	0	0	0
	2	10	10	10	2	2	1	0	0	0
	3	10	10	7	5	0	0	0	0	0
	4	10	10	3	1	1	0	0	0	0
	5	10	10	5	1	0	0	0	0	0
		100%	100%	66%	20%	8%	2%	0%	0%	0%
4.0 ppb	1	10	9	2	1	0	0	0	0	0
	2	10	9	4	1	0	0	0	0	0
	3	10	9	2	0	0	0	0	0	0
	4	10	9	3	0	0	0	0	0	0
	5	10	9	3	1	0	0	0	0	0
		100%	90%	28%	6%	0%	0%	0%	0%	0%
11.0 ppb	1	10	0	0	0	0	0	0	0	0
	2	10	0	0	0	0	0	0	0	0
	3	10	0	0	0	0	0	0	0	0
	4	10	0	0	0	0	0	0	0	0
	5	10	0	0	0	0	0	0	0	0
		100%	0%	0%	0%	0%	0%	0%	0%	0%

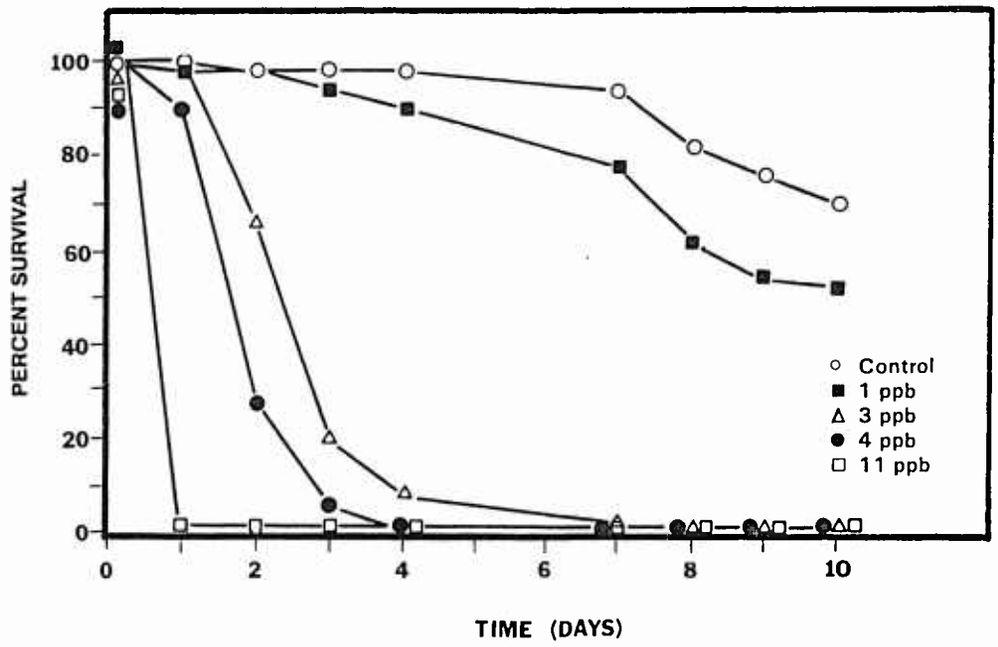

Figure 17. Survival of subadult mysids exposed to TBTO for 10 days in June 1981.

Table 18. Survival of *Metamysidopsis elongata* (mysids) exposed to monobutyl-, dibutyl-, and tributyltin (17 May 1983). Butyltin concentrations were measured by GF-AAS analysis.

		Time (Days)				
Treatment	Replicate	0	1	2	3	4
Control	1	11	10	7	7	7
	2	11	11	11	10	8
	3	10	9	9	9	6
	4	10	10	10	10	9
	5	10	10	10	9	7
		100%	96%	90%	86.5%	71%
MBTCL	1	10	10	9	8	7
16 ppb	2	10	10	10	9	6
	3	10	10	10	10	7
	4	10	10	10	8	6
	5	10	10	9	8	5
		100%	100%	96%	86%	62%
MBTCL	1	11	10	8	8	6
161 ppb	2	11	9	9	8	6
	3	10	9	9	8	6
	4	10	10	10	9	7
	5	10	10	10	9	8
		100%	94%	90%	82%	65%
MBTCL	1	10	10	10	10	6
809 ppb	2	9	9	9	9	7
	3	10	10	10	10	10
	4	10	10	9	9	6
	5	10	10	10	7	4
		100%	100%	98%	92%	67%

Table 18. Survival of *Metamysidopsis elongata* (mysids) exposed to monobutyl-, dibutyl-, and tributyltin (17 May 1983). Butyltin concentrations were measured by GF-AAS analysis (continued).

		Time (Days)				
Treatment	Replicate	0	1	2	3	4
Control	1	11	10	7	7	7
	2	11	11	11	10	8
	3	10	9	9	9	6
	4	10	10	10	10	9
	5	10	10	10	9	7
		100%	96%	90%	86.5%	71%
TBTCL	1	10	10	9	9	8
0.75 ppb	2	10	10	10	9	5
	3	10	10	10	6	3
	4	10	10	10	8	9
	5	12	12	12	11	8
		100%	100%	98%	84%	60%
TBTCL	1	11	11	11	10	9
1.5 ppb	2	14	14	14	14	11
	3	10	10	10	9	8
	4	11	11	10	10	7
	5	12	12	11	8	8
		100%	100%	96.5%	88%	74%
TBTCL	1	13	12	11	4	1
6.0 ppb	2	11	10	7	3	2
	3	12	12	9	6	3
	4	14	14	14	6	3
	5	13	9	5	5	3
		100%	90%	73%	38%	19%

Table 18. Survival of *Metamysidopsis elongata* (mysids) exposed to monobutyl-, dibutyl-, and tributyltin (17 May 1983). Butyltin concentrations were measured by GF-AAS analysis (continued).

		Time (Days)				
Treatment	Replicate	0	1	2	3	4
Control	1	11	10	7	7	7
	2	11	11	11	10	8
	3	10	9	9	9	6
	4	10	10	10	10	9
	5	10	10	10	9	7
		100%	96%	90%	86.5%	71%
DBTCL	1	10	10	10	10	8
2 ppb	2	10	9	9	8	3
	3	10	10	9	9	6
	4	10	10	10	10	10
	5	10	10	10	9	9
		100%	98%	96%	92%	72%
DBTCL	1	10	10	10	10	9
11 ppb	2	10	9	9	7	6
	3	10	10	10	10	7
	4	10	9	9	8	6
	5	10	10	9	9	6
		100%	96%	94%	88%	68%
DBTCL	1	10	10	9	7	4
56 ppb	2	10	10	8	7	1
	3	10	10	8	3	1
	4	10	10	10	9	5
	5	10	10	10	9	5
		100%	100%	90%	70%	32%

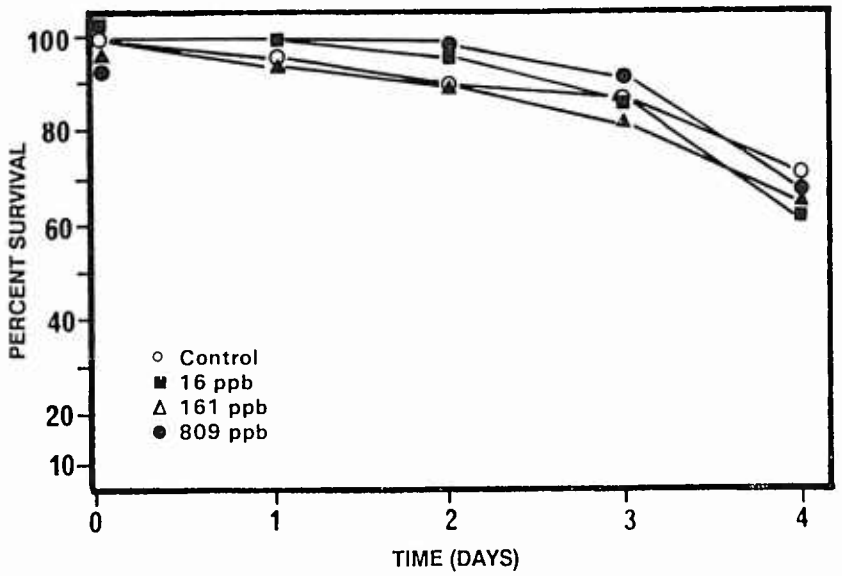

Figure 18a. Survival of mysids exposed to monobutyltin in May 1983.

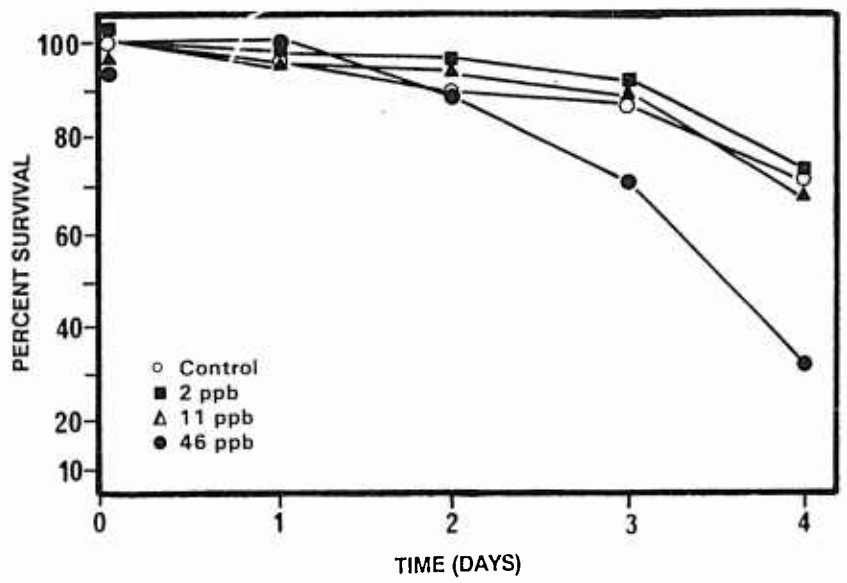

Figure 18b. Survival of mysids exposed to dibutyltin in May 1983.

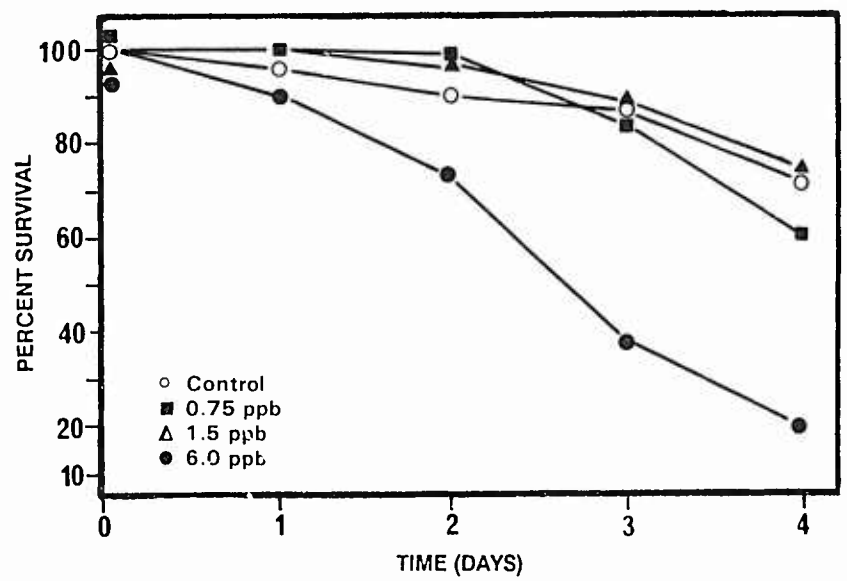

Figure 18c. Survival of mysids exposed to tributyltin in May 1983.

Table 19. Survival of *Acartia tonsa* (copepods) exposed to TBTO (30 March 1981). TBTO concentrations were nominal.

		Time (Days)				
Treatment	Replicate	0	1	2	3	4
Control	1	10	9	8	6	6
	2	10	9	7	5	5
	3	10	9	9	9	8
		100%	90%	80%	66%	63%
1.0 ppb	1	10	0	0	0	0
	2	10	0	0	0	0
	3	10	0	0	0	0
		100%	0%	0%	0%	0%
3.0 ppb	1	10	0	0	0	0
	2	10	0	0	0	0
	3	10	0	0	0	0
		100%	0%	0%	0%	0%
15.0 ppb	1	10	0	0	0	0
	2	10	0	0	0	0
	3	10	0	0	0	0
		100%	0%	0%	0%	0%

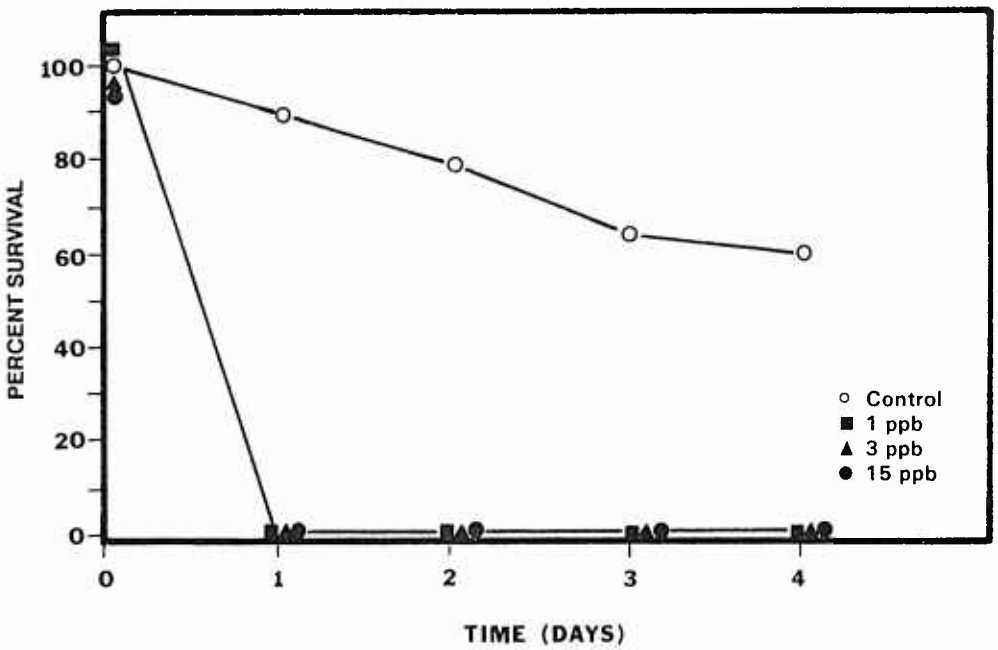

Figure 19. Survival of copepods exposed to TBTO for 4 days in March 1981.

Table 20. Survival of *Acartia tonsa* (copepods) exposed to copper (30 March 1981). Copper concentrations were nominal.

		Time (Days)				
Treatment	Replicate	0	1	2	3	4
Control	1	10	9	8	6	6
	2	10	9	7	5	5
	3	10	9	9	9	8
		100%	90%	80%	66%	63%
10.0 ppb	1	10	8	7	7	4
	2	10	8	6	6	3
	3	10	8	6	6	4
		100%	80%	63%	63%	36%
50.0 ppb	1	10	9	7	7	3
	2	10	6	4	3	3
	3	10	8	6	2	2
		100%	76%	56%	46%	26%
250.0 ppb	1	10	4	1	0	0
	2	10	5	1	1	0
	3	10	7	1	0	0
		100%	53%	10%	3%	0%

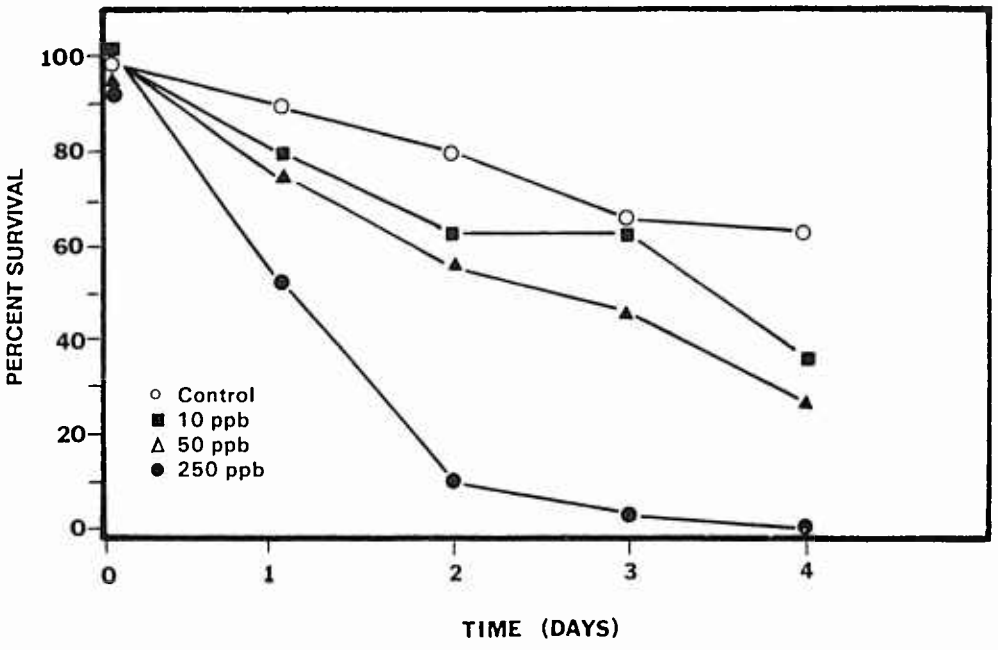

Figure 20. Survival of copepods exposed to copper for 4 days in March 1981.

Table 21. Experimental conditions used for the various organotin tests.

	Concentration of Toxicant (ppb)			Tank Size	Static/ S-Renewal	Duration (Days)	Fed	#/ Rep	# Reps
P. staminea									
TBTO (1/81)	3	15	76	16 L	Static	10	No	10	3
TBTO (4/81)	40	119	271	16 L	Static	13	No	10	3
Copper (4/81)	250	750	2250	16 L	Static	14	No	10	3
M. edulis									
TBTO (1/81)	3	15	76	16 L	Static	10	No	10	3
C. stigmaeus									
TBTO (4/81)	3	19	123	16 L	Static	14	No	10	3
Copper (4/81)	250	750	2250	16 L	Static	14	No	10	3
N. arenaceodentata									
TBTO (4/81)	10	35	150	1 L	Static	6	No	10	3
Copper (4/81)	250	750	2250	1 L	Static	6	No	10	3
TBTO (7/81)* 4	10	14	20	1 L	Static	6	No	10	5
M. elongata									
TBTO (1/81)	1	3	14	1 L	Static	7	Yes	10	3
TBTO (4/81)	2	10	14	1 L	Static	5	Yes	10	3
Copper (4/81)	10	30	90	1 L	Static	7	Yes	10	3
TBTO (7/81) 0.2	1	3	8	1 L	Static	4	Yes	10–12	5
TBTO (11/81)	0.25	1	4	1 L	S-Renewal	6	Yes	10	5
SPC (11/81)	0.25	1	4	1 L	S-Renewal	6	Yes	10	5
TBTO (2/81)*	1	4	22	1 L	Static	7	Yes	9	3
TBTO (6/81)* 1	3	4	11	1 L	Static	10	Yes	10	5
MBTCL (5/83)	16	160	809	1 L	Static	4	Yes	10–11	5
DBTCL (5/83)	2	11	56	1 L	Static	4	Yes	10–11	5
TBTCL (5/83)	0.75	1.5	6.0	1 L	Static	4	Yes	10–11	5
A. tonsa									
TBTO (3/81)	1	3	15	400 ml	Static	4	No	10	3
Copper (3/81)	10	50	250	400 ml	Static	4	No	10	3

*Subadults or juveniles used.

Table 22. Summary of organotin measurement data. Concentrations are in ppb.

Test Organism	Date	Nominal Concentration	Time of Measurement (Days) 0	1	2	3	4
Clam	1/81	40.0					2.0
		119.0		59.0	45.0	45.0	20.0
		271.0				105.0	
Mussel	1/81	15.0		13.0			4.0
		76.0		52.0			8.0
Blank		15.0				8.0	
		76.0				39.0	
Fish	4/81	3.0				ND*	
		19.0		14.0	2.0	ND*	
		123.0				12.0	
Worm	4/81	35.0		18.0	31.0	25.0	7.9
		150.0				80.0	
Juvenile Worm	7/81	4.0		4.0	4.0	2.0	2.0
		10.0		11.0	10.0	6.0	5.0
		14.0		15.0	14.0	9.0	9.0
		20.0		17.0	15.0	13.0	
Mysid	1/81	15.0		9.0			4.0
Mysid	4/81	4.0					
		10.0				9.0	
		14.0		11.0	12.0		6.0
Mysid	11/82	0.25	0.3	0.2			
		1.0	1.7	2.5	2.2	1.6	
		4.0	4.5	4.7	4.8	4.8	
Mysid - SPC test	11/82	0.25	0.3	0.2			
		1.0	1.6	2.9	2.4	2.0	
		4.0	4.2	5.1	4.4	5.2	

*ND = Nondetectable by analytical method used.

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APPENDIX A

EFFECTS OF ORGANOTINS ON ORGANISMS: A LITERATURE SURVEY

ABBREVIATIONS USED IN APPENDIX I

$BTCl_3$	Butyltin chloride
$DBTCl_3$	Dibutyltin chloride
$DETCl_2$	Diethyltin chloride
$DMTCl_2$	Dimethyltin chloride
$DPTCl_2$	Diphenyltin chloride
$MTCl_3$	Methyltin chloride
$PTCl_3$	Phenyltin chloride
TBT-	Tributyltin
TBTA	Tributyltin acetate
TBTB	Tributyltin benzoate
TBTCl	Tributyltin chloride
TBTF	Tributyltin fluoride
TBTL	Tributyltin laurate
TBTM	Tributyltin methyl
TBTO	Tributyltin oxide
TBTOl	Tributyltin oleate
TETO	Triethyltin oxide
TETCl	Triethyltin chloride
TETS	Triethyltin sulfate
TMTCl	Trimethyltin chloride
TMTO	Trimethyltin oxide
TPTA	Triphenyltin acetate
TPTCl	Triphenyltin chloride
TPTF	Triphenyltin fluoride
TPTO	Triphenyltin oxide
TPTOH	Triphenyltin hydroxide
TPrTCl	Tripropyltin chloride
TPrTO	Tripropyltin oxide

REFERENCE	CMPD	ORGANISM	METHODS	RESULTS
			PART I: MAMMALS	
Polster (1970)	TBTO TBTA TBTCl TBTOl TBTL TBTB	Mammals		LD-50: 100-250 mg/kg
Sheldon (1975)	TBTO TBTF TPTF	Rats Rabbits	Noted animal skin irritation Applied tins to skin	LD-50 (mg/kg) IRRITANT TO RABBITS RATS / RABBITS / SKIN / EYE TBTO 234 / 11,700 / Severe / Extreme TBTF 200 / 680 / Minimal / Extreme TPTF 1170 / 1,000 / Minimal / Extreme
			Organotins combined with paint	TBTO -- / -- / Moderate / Severe TBTF 4556 / 10,250 / Extreme / Extreme TPTF -- / -- / Moderate / Extreme
			90-day sub-acute toxicity studies with TBTF	1) 68.0 mg/kg is toxic 2) 14.0 mg/kg is a no-effect level 3) toxic effects appear reversible
		White Mice	Leukocyte counts, blood urea N_2 concentration, fasting blood glucose concentration, microscopic skin changes, carcinogenicity study (male white mice): 6 mos old, 15 mg of 5-10% TBTF 3 times/ week for 6 mos.	No carcinogenic Effects
Stoner (1966)	TPTA	Rats, Mice, Guinea pigs Rabbits, Hens	Acute oral toxicity w/feeding and intraperitoneal administration Some skin application studies	Guinea pig most sensitive. Growth was inhibited @ 1 ppm in diet. TPTA did not readily penetrate unbroken skin.
Tan & Ng (1977)	TMTCl TETS	Animal tissues	Toxicity assessed by effects on isolated phrenic nerve-diaphragm preparations. Electron microscope studies.	Inhibitory effects on muscular (1977) contractility of tissue are associated with a) disruption of mitochondria & disorganization of muscle fibres, b) depletion of neuronmicrotubules in axons of nerves innervating the muscle. Neurotoxic effect: inhibition of the specific colchicine-binding activity of crude & purified tubulin preparations from brain tissue. TMTCL & TETS conc > 100 uM completely prevented normal *in vitro* assembly of microtubules from tubulin.

REFERENCE	CMPD	ORGANISM	METHODS	RESULTS
		PART II: VERTEBRATES (EXCLUDING MAMMALS)		
Alabaster (1969)	TBTO	Salmo gairdneri		24-hr LC-50: 0.028 mg/l 48-hr LC-50: 0.021 mg/l
Berrios-Duran & Ritchie (1968)	TBTO	Micropterus Leponis		No mortality after 25 days following a toxic release of 0.5 mg TBTO/l over a 3-day period.
Cardarelli (1973)	TBTO	Lebistes reticulatus	Added TBTO in one dose at the beginning of the experiment.	30-day LC-50: 0.007 mg/l 90-day LC-50: 0.004 mg/l
Cardarelli (1974)	TBTO	Lebistes reticulatus	Added TBTO daily in test tanks simulating micro-environments.	2% lethality after 120 days at 0.007 mg/l/day.
Chliamovitch & Kunn (1977) epithelium. mainly	TBTO	Salmo gairdneri Tilapia rendalli	96-hr Tests 11.7 ug/l to 5.85 mg/l tested. Fish not fed during test.	24-hr EC-50: 30.8 ug/l for S. gairdneri 53.2 ug/l for T. rendalli 0.0117 to 5.85 mg TBTO/l resulted in damage to gill 1.17 mg/l resulted in cell swelling followed by death. 0.053 mg/l resulted in cell shrinkage. TBTO interferred with respiration. 11.7 ug/l: only 10-20% survival after returning fish to uncontaminated tanks. "Safe level" = 1.0 ug/l
Deschiens et al. (1966)	TBTO	Tilapia nilotica		Application of 0.03 mg/l caused no effects after 15 days.
Dundee, Giardina, Swindler & Good (1980)	TBTA TPTA TBTO TPTO TPTOH TPTCL TPTF	Arius felis, Anchoa mitchilli, Menidia beryllina, Micropogon undulatus, Mugil cephalus	Painted panels, organotin added to water, solid substrate test = percolating 2 liters of 3.5 ppb organotin seawter thru substrate, then used test water.	Leachate study: Order of toxicity = TBTAC = TPTAC > TBTO = TPTO > TPTOH > TPTCl = TPTF. Substrate Study: substrate removed organotin from habitat water up to a saturation point; saturation does not detoxify compounds. Fish demonstrated stress within 20 minutes for concentrations > 0.10 ppm

REFERENCE	CMPD	ORGANISM	METHODS	RESULTS
Dundee, Giardina, Swindler & Good (1980)			Comparative Survival Times	Survival Species — Cmpd — Max Days Gambusia affinis — TBTA — 2-5 Poecillia latipinna — TBTA — 0.8-0.9 Cyprinodon varigatus — TBTO — 2-3 Menidia berylina — TBTO — 0.08-0.8 Fundulus grandis — TPTCl — 4-5 Micropogon undulatus — TPTCl — 2-5 Crassostrea virginica — TBTA -2% — 15-18 -10% — <3 TBTO -2% — <9 -10% — <6 TPTCl -2% — 18-22 -10% — 8-10 Rangia cuneata — TBTA -2% — 6-13 TBTA -10% — 4-6 TBTO -2% — 8 TBTO -10% — 5-6 TPTCl -2% — 12 TPTCl -10% — 6-13 Test panels showed each organotin cmpd to be toxic to all organisms except the TPTCl which was fatal to fish within 4 days, was not toxic to oysters during the 22 days.
Floch et al. (1964)	TBTO	Rana temporaria Carassius auratus Lebistes		24-hr LC-100: 0.075 mg/l Concluded that TBTO is non-selective.
Good, Dundee & Swindler (1980)	TBTA	Fish	3 liters lake water + 0.02 - 1.33 ppm organotin added.	0.02 - 1.0 ppm = in 100% mortality within 20 min to 3 hrs. 0.10 ppm resulted in 100% mortality within 24 hours 0.02 ppm resulted in 100% mortality within 12 days Fish distress symptoms: erratic swimming; loss of equilibrium & orientation; convulsions; loss of buoyancy
Linden et al. (1979)	TBTF TBTO TPTF	Alburnus alburnus Nitocua spinipes	Acute mortality tests under static conditions.	96-hr LC-50 (mg/l) — TBTF — TBTO — TPTF A. alburnus: — 0.006-0.008 — 0.002 — 0.400 N. spinipes: — 0.015 — 0.002 — 0.008
Matthiessen (1974)	TBTO	Tilapia mossambica		24-hr LC-50: 0.028 mg/l
Polster &	TBTO	Lebistes		7-day LC-50: 0.040 mg TBTO/1

REFERENCE	CMPD	ORGANISM	METHODS	RESULTS
Polster & Halacka (1971)	TBTO	Lebistes reticulatus		7-day LC-50: 0.040 mg TBTO/1
Schatzberg & Harris (1979)	Various	Guppy fry Goldfish fry	20 gm of grit (used to remove paints from ship hulls) shaken w/300 ml water for 24 hours; water decanted; fish added to water; observed for 24 hours.	Guppy fry (After 24 Hours Exposure) 75% survival @ 5 ppb Sn 50% survival @ 10 ppb Sn 0% survival @ 20 ppb Sn Goldfish fry (After 24 hours) 100% survival @ 5 ppb Sn 100% survival @ 10 ppb Sn 50% survival @ 20 ppb Sn 0% survival @ 30 ppb Sn
Stroganov, et al. (1973)	TETCl	Carp 1 yr old	TETCl tagged w/Sn-117, added to tanks 2 & 6 day tests: 1 mg/l 15, 30, 45 day tests: 0.1 & 0.01 mg/l. Counted activity in all tissues. No feeding occurred	TETCl accumulated in organs and tissues. Maximum in bile and blood. Minimum in backbone and white muscles.
Swindler, Dundee & Good (1979)	TPTA TPTOH TPTCl TPTO TPTF	Fish	Aluminum panels coated with organotin; 42-day leachate test	No deaths with TPTF, TPTCl or TPTO; 100% mortality w/in 22 days with TPTA and TPTOH. Fish distress symptoms could be reversed by placing orgnaisms in clean tanks with fresh water.
			Water from tank with either sand or mud substrate (19-day)	Mud: 100% Survival Sand: Most died within 20 days.
Ward et al. (1981)	TBTO	Sheepshead minnows Cyprinodon variegatus	Studied acute & chronic toxicity 0.33 - 3.2 ppb -C^{14} labeled TBTO Studied life cycles: Part I: 0.34 - 4.8 ppb TBTO Part II: 0.14 - 0.45 ppb TBTO	Maximum bioconcentration factor = 2600x for whole fish: Muscle = 1810x, viscera = 4580x, remains = 2120x, liver = 20-52,000x Release of C^{14} labeled TBTO: 52% after 7 days, 74% after 28 days. 21-day LC-50 = 0.003 mg/l TBTO 100% mortality @ 3.2 ppb. Life Cycle Study: Parental fish - 100% mortality @ 4.8 ppb within 4 days. After 167 days, no difference between controls and treatments in growth, fecundity, hatching success (< 0.45 ppb) or time of hatching, or juvenile mortality (< 0.45 ppb); however, possible juvenile mortality @ 0.45 ppb.

REFERENCE	CMPD	ORGANISM	METHODS	RESULTS
Weisfeld (1970)	TBTO TBTM	Guppies	Wooden depressors coated w/paints containing organotins, 8 liter aquaria with 8-11 fish/tank, 4 day test, fish fed during test.	%Mortality/Time Test #1 / Test #2 Cu_2O/7% TBTO: 100%/72 hrs / 100%/50 hrs Cu_2O/7% TBTM: 38%/72 / 40%/72 7% TBTO: 100%/48 / 100%/48 7% TBTM: 89%/24 / 40%/79 10% TBTO: 100%/24 / 100%/24 10% TBTM: 100%/24 / 100%/24
PART III: MICROORGANISMS				
Hallas & Cooney (1981a)	18 various cmpds.	11 Microbial isolates from Chesapeake Bay	Growth of isolated on medium containing organotins	TETCl effective against all isolates. Tri-organotins most effective, Di- and some mono-organotins almost inhibitory. Tetraalkyltins least toxic. The 11 isolates exhibited a diversity of sensitivity patterns to the organotin compounds tested.
Hallas & Cooney (1981b)	DMTCl	Microbial flora from Chesapeake Bay	Examined sediment & water samples from Chesapeake Bay for microbial populations resistant to DMTCl. Determined conc of tin in water & sediment.	Microbial flora more sensitive to DMTCl than inorganic tin. 15 mg Sn/l as DMTCl significantly reduced microbial counts.
Polster (1970)	TBTO TBTA TBTCl TBTOl TBTL TBTB	Bacteria, fungi, phytoplankton		LD-50 ranged between 0.082-0.10 mg/l.
Sijpestienj et al. (1964)	Various	Aspergillus niger and other fungi	Glucose agar - growth inhibition after 3 days	Minimum inhibitory concentration ug/ml Cmpd (M = Sn) Type / R= Ethyl / Butyl / Phenyl R_4M: >500 / >500 / >500 R_3MX: 2 / 1 / 0.5 R_2MX_2: >500 / >500 / 10 RMX_2: >500 / >500 / 500 -R3MX is most powerful fungicide -Trialkyltin cmpds strong inhibitors of oxidative phosphorylation in mitochondira of animal tissues -Gram negative bacteria are less sensitive to organotins -Gram positive are more sensitive

REFERENCE	CMPD	ORGANISM	METHODS	RESULTS
			PART IV: PLANTS	
Evans & Smith (1975)	R_3SnX	Algae	Reporting work of Phillip (1973)	Range of Activity (ppm) R_xSnX 0.01 - 5 Cu_2O 1 - 50 R_3PbX 0.1 - 1 RHgX 0.1 - 1
Kahn (1968)	Chlorotri-n-butyltin	Euglena gracilis Spinach	Isolated chloroplasts, assay for photophosphorylation in the presence of tin.	Tin found to inhibit photophosphorylation at extremely low concentrations; ratio of chlorophyll to tin major determinant of the degree of inhibition. Euglena: 1 mole tin/120 moles Chloro Spinach: 1/60
Smith & Burton (1972)	Various	Marine brown algae	Tissues digested under reflex, con sulfuric acid (ambient levels determined)	Desmarestia aculata: 0.65 ppm Sn/dry wt Laminaria sacharina: 0.29 L. digitata: 0.13 Dictyota dichotoma: 0.10
		Phytoplankton	Collected in Ocean Water	Mixed species: 3.5 ppm dry wt Sn Content
Stroganov et al. (1970)	Various	Chlorella vulgaris Scenedesmus quadricaudi Daphnia magna		Organotin toxic at 0.02 - 0.5 mg/l
Stroganov et al. (1973)	Trialkly tins	Phytoplankton Zooplankton	Collected in Lakes	Organisms disappear at concentrations as low as 0.1 mg/l.

REFERENCE	CMPD	ORGANISM	METHODS	RESULTS
Wong et al. (1982)	Various	Scenedesmus quadricauda, Anabena flos-aquae, Ankistrodesmus falcatus Lake Ontario Algae	Primary productivity w/C^{14}, reproduction experiments	S. quadricauda: TBTO: 4-hr IC50 = 0.016 mg/l TPTCl: 48-hr IC50 = 0.040 mg/i TETCl: 4-hr IC50 = 0.100 mg/l TMTCl: 4-hr IC50 = 2.600 mg/l $DMTCl_2$: 4-hr IC50 = 0.020 mg/l 8-day IC50 = 0.005 mg/l A. falcatus: TBTO: 4-hr IC50 = 0.020 mg/l 8-day IC50 = 0.005 mg/l TPTCl: 4-hr IC50 = 0.010 mg/l 8-day IC50 = 0.002 mg/l TPrTCl: 4-hr IC50 = 0.020 mg/l 8-day IC50 = 0 014 mg/l TETCl: 4-hr IC50 = 0.200 mg/l 8-day IC50 = 0.100 mg/l TMTCl: 4-hr IC50 = 5.500 mg/l 8-day IC50 = 0.570 mg/l DMTCl: 4-hr IC50 = 21.00 mg/l $DETCl_2$: 4-hr = 16.00 mg/l $DBTCl_2$: 4-hr IC50 = 6.80 mg/l $DPTCl_2$: 4-hr IC50 = 8.00 mg/l $MTCl_3$: 4-hr IC50 = 23.00 mg/l $BTCl_3$: 4-hr IC50 = 25.00 mg/l $PTCL_3$: 4-hr IC50 = 19.00 mg/l A. flosaquae: TMTCl: 4-hr IC50 = > 5.00 mg/l TBTO: 4-hr IC50 = 0.013 mg/l TPTCl: 4-hr IC50 = 0.020 mg/l $DMTCL_2$: 4-hr IC50 = > 5.00 mg/l Lake Ontario Algae: TMTCl: 4-hr IC50 = 0.350 mg/l TETCl: 4-hr IC50 = 0.055 mg/l TPrTCl: 4-hr IC50 = 0.004 mg/l TBTO: 4-hr IC50 = 0.003 mg/l TPTCl: 4-hr IC50 = 0.002 mg/l In general, indigenous algae appeared more sensitive to tin compounds than pure algae. Triphenyl & tributyl comps were toxic at 0.1 mg/l.

REFERENCE	CMPD	ORGANISM	METHODS	RESULTS
			PART V: INVERTEBRATES	
Boulton *et al.*	TETS	*Elminus*	Investigated metabolism of glucose & acetate inpresence of TETS. Used radiocarbons.	Some results may be related to uncoupling of oxidative phosphorylation. Some disruption of pyruvate metabolism *in vivo*. Major effect *in vivo* is to increase % incorporation from isotopically labelled glucose & pyruvate into alanine & lactic acid, and decrease recovery of radiocarbon in glutamic acid and glutamine. TETS did not inhibit any of the enzymes tested sufficiently to strongly account for its toxicity.
Cardarelli (1973)	TBTO	*Biomphalaria glabrata*		30-day LC-99 = 0.0004 mg/l
Cardarelli (1974)	TBTF	*Biomphalaria glabrata*		30-day LC100 = 0.014 mg/l 60-day LC100 = 0.007 mg/l 90-day LC100 = 0.0035 mg/l
Deschiens *et al.* (1966)	TBTO	*Biomphalaria glabrata*		5-day LC-100 = 0.015 mg/l
Dundee, Giardina, Swindler & Good (1980)	TBTA TPTA TPTOH TBTO TPTO TPTCl TPTF	*Crassostrea virginica* *Ischadium recurvum* *Neritina reclivata* *Callinectes sapidus* *Clibanarus bivittatus* *Paleomonetes* sp. *Penaeus setiferus* *Penaeus aztecus*	Painted panels; organotin added to water, solid substrate test consisted percolating 2 liters of 3.5 ppb organotin seawater thru test water.	Molluscs survived longer, but all eventually died if initial organotin concentration was >0.04 ppm. Oysters exposed to TPTA concentrations of 0.01, 0.02 and 0.04 ppm normal and the filed shell edges were redeposited after 8 days. Crustaceans more resistant. *C. sapidus* able to survive 1.0 ppm for 10 days before death. *Species* — LC — Max Surv Conc *C. sapidus* — >1.0 ppm — <0.2 ppm *C. bivittatus* — >0.2 ppm — <0.18 ppm *P. aztecus* — >0.1 ppm — <0.08 ppm *P. setiferus* — >0.1 ppm — <0.08 ppm *Paleomontes* sp — >0.1 ppm — <0.08 ppm

REFERENCE	CMPD	ORGANISM	METHODS	RESULTS
Evans & Smith (1975)	R_3SnX	Barnacles	Reporting work of Phillip (1973)	Range of Toxicity (ppm) R_3SnX 0.1 - 1 Cu_2O 1 - 10 R_3PbX 0.1 - 1 RHgX 0.1 - 1
Filenko & Isakova (1979)	TPTCl	Daphnia magna	0.01, 0.05, 0.1 mg/l Accumulation studied. Concentration in water changed with time.	Concentrates TPTCl in body by means of adsorption through body surface.
Floch et al. (1964)	TBTO	Bulinus contortus Australorbis glabratus Daphnia longispina		24-hr LC-100 = 0.075 mg/l 5-day LC-100 = 0.03 mg/l 24-hr LC-100 = 0.12 mg/l
		Cypridopsis hartwigi		TBTO: 24-hr LC100 = 4 mg/l 48-hr LC100 = 2 mg/l 4-day LC100 = 0.12 mg/l TBTA: 24-hr LC100 = 2 mg/l 48-hr LC100 = 1 mg/l 4-day LC100 = 0.15 mg/l
Frick & de Jimenez (1964)	TPrTO TBTA TPTA	Australorbis glabratus	1-6 hr & 4 day old incubated eggs 24 hr old snails, 3-5, 8-10 and 13-15 mm snails. All exposed for 6-24 hours. Mortality determined after 24 hour recovery period.	SEE BELOW

CONCENTRATION OF TOXICANT (ppm) PRODUCING EFFECTS AFTER 24 HOURS

Toxicant	Effect	EGGS 1-6 hr.	EGGS 4 day	SNAILS Newly Hatched	SNAILS 3-5 mm	SNAILS 8-10 mm	SNAILS 13-15 mm
TBTA	LC-50	0.031	0.23	0.019	0.041	0.074	0.069
LC-90	0.072	0.45	0.041	0.056	0.105	0.115	
	100% Mort.	0.2	0.8	0.1	0.1	0.2	0.2
TPrTO	LC-50	0.06	0.099	0.03	0.029	0.043	0.085
	LC-90	0.117	0.17	0.049	0.033	0.078	0.115
	100% Mort.	0.2	0.2	0.1	0.04	0.2	0.2
TPTA	LC-50	0.12	1.6	0.07	0.305	0.65	0.66
	LC-90	0.22	3.5	0.115	0.44	0.84	1.55
	100% Mort.	0.5	4.0	0.2	0.5	0.9	4.0

CONCENTRATION OF TOXICANT (ppm) PRODUCING EFFECTS AFTER 6 HOURS

Toxicant	Effect	EGGS 1-6 hr.	EGGS 4 day	SNAILS Newly Hatched	SNAILS 3-5 mm	SNAILS 8-10 mm	SNAILS 13-15 mm
TBTA	LC-50	0.094	0.56	0.074	0.088	0.2	0.19
	LC-90	0.17	1.17	0.185	0.14	0.38	0.3
	100% Mort.	0.4	1.6	0.2	0.2	0.6	0.4
TPrTO	LC-50	0.11	0.21	0.275	0.15	0.38	0.78
	LC-90	0.26	0.37	0.53	0.22	0.57	1.05
	100% Mort.	0.4	0.8	0.8	0.4	0.8	1.6
TPTA	LC-50	0.37	2.3	0.49	1.15	1.35	*
	LC-90	0.78	5.0	1.0	1.65	2.35	*
	100% Mort.	2.0	8.0	2.0	2.0	6.0	*

* Not included in test.

REFERENCE	CMPD	ORGANISM	METHODS	RESULTS
Good, Dundee & Swindler (1980)	TBTA	Rangia cuneta	3 liters lake water & organotin: concentrations of 0.02 - 1.33 ppm studied. No substrate added.	Adults: LD = 0.10 ppm, death in 8-17 days. 1.0 ppm: 100% mortality within 5 days. 0.02 ppm: 100% survival Symptoms of Toxicity: siphons not fully extended; excess mucus; cloudy water (from mucus); swollen siphons & mantle insensitive to touch. Juveniles: 0.04 - 0.09 ppm: 100% mortality within 6 days. LD < 0.02 ppm. Same distress symptoms as adults.
Hopf et al. (1967)	TBTA TBTO TPrTO	Biomphalaria glabrata Lebistes Mice		B. glabrata adults: TPTA: 24 hr exposure @ 0.05 ppm with a 120 hr recovery gave a 50% kill. Lebistes: TPTOH: 0.1 ppm killed 43% after 19 hrs 0.1 ppm killed 100% after 48 hrs 0.1 ppm killed 100% after 72 hrs 0.1 ppm killed 100% after 96 hrs Mice: TPTOH: 14-day LD-50 = 245 mg/kg
Laughlin & French (1980)	TBTO TPTO TETO TMTO TPrTO	Larvae of: Hemigrapsus Homarus americanus	14-day tests, larvae counted daily, live transferred to new tanks at respective concentrations Hemigrapsus exposed to: TBTO & TPrTO: 25, 50, 75, 100, 500 and 1000 ppb. TETO & TMTO: 50, 75, 100, 150, 500 and 1000 ppb. H. americanus exposed to: TBTO: 0, 1, 2, 10, 15 and 20 ppb	Hemigrapsus: After 14 days: Controls = 80-84% All Alkyltins lethal 500 & 1000 ppb conc had no survival beyond 2 days, 25-100 ppb resulted in 100% mortality by day 8. TPrTO: 500 & 1000 ppb exposure resulted in 100% mortality after 2 days. Dose dependent mortality between 2-6 days for lower concentrations. TETO: Most toxic compound. 150 ppb exposure resulted in 50% survival after 2-8 days; no survival beyond 8 days at lower concentrations. H. americanus were more sensitive, 20 ppb resulted in 100% mortality after 24 hours. 5-15 ppb resulted in 2-6 days dose-dependent mortality.

REFERENCE	CMPD	ORGANISM	METHODS	RESULTS
Laughlin & Guard (1981)	TBTO TBTF	Orchestoidea californiana	Organotin solutions prepared in acetone: 0.5, 1, 3, 6, 10 and 15 ppb concentrations. 9-day exposure to organotins. TBTO concentration measured with gas chromatograph.	TBTO (survival): Control: 80% 0.5 ppb: 87% 1, 3, 6 ppb: 53% (Note: not consistently dose dependent) 10 ppb: 20% 15 ppb: 7% TBTF (survival): Control: 80% <6 ppb: >50% (Not dose dependent) 10 ppb: 13% 15 ppb: 0%
Polster & Halacka (1971)	TBTO	Daphnia magna Tubifex tubifex		48-hr LC-50 = 0.003 mg/l 48-hr LC-50 = 0.006 mg/l
Smith & Burton (1972)	Various	Marine Gastropods & bivalves	Tissue digestions	Crepidula fornicata: 0.71 ppm dry wt Buccinum undatum: 0.33 ppm dry wt Cardium edule: 0.67 ppm dry wt Mercenaria mercenaria: 0.23 ppm dry wt
Smith et al. (1979)	TBT-	Biomphalaria glabrata		

Compound	LC-50 (mg/l)
TBTO	0.05 - 0.1
Bu_3Sn-O-CO-(3-pyridyl)	0.01 - 0.05
Bu_3Sn-O-CO-(2-pyridyl)	0.01 - 0.05
Bu_3Sn-O-Ch2-Ch2-NEt_2	0.1 - 0.25
Bu_3Sn-S-Ch_2-CO-O-(2-ethylhexyl)	0.05 - 0.1
Bu_3Sn-O-SO_2-Me	0.01 - 0.05
$Bu_3Sn(Ph_3PO)_2$ BPh_4	0.05 - 0.1
Bu_3SnCl_2 Ph-CH_2-PPh_3	0.1
Bu_3Sn-O-CO-ME	0.1 - 0.3
Bu_3Sn-NCS, Ph_3AsO	0.05 - 0.1
$BuPh_2Sn$-O-CO-Me	0.05 - 0.1
Ph_3Sn-O-CO-Me	0.05
$BuPh_2SnPh_3PO_2$ BPh_4	0.1 - 0.2
$BuPh_2Sn$-OH	0.05 - 0.1
$BuPh_2Sn$-Br	0.05 - 0.1

Stroganov et al. (1973)	TETCl	Molluscs Daphnia		Lethal @ 0.2 mg/l Lethal @ 0.005 mg/l

REFERENCE	CMPD	ORGANISM	METHODS	RESULTS
Stroganov et al. (1977)	Various	Lymnaea stagnalis		Minimum lethal concentration = 0.1 mg/l At concentrations between .000001 - .010 mg/l food assimilation diminished, soft body either hydrated or dehydrated, shell thin, reproduction supressed.
Swindler Dundee & Good (1979)	TPTA TPTOH TPTCl TPTO TPTF	Clams Mussels Oysters	Coated aluminum panels with o-tins 42 day leachate study	Clams & mussels: no deaths with TPTF, TPTCl or TPTO; 100% mortality within 22 days for TPTA and TPTOH.
			1 liter substrate & water allowed to stand for 19 days, water drained and used for tests.	Mud: 100% survival Sand: Most died within 20 days
			42-day leachate study, filed shells of oysters to allow constant exposure to contaminated water.	Controls and 0.04 ppb TPTA rebuilt shells in 8 days. At concentrations >0.06 ppm TPTA, no rebuilding of shell, greater mucus production.
Young & Alexander (1977)	Various	Mytilus edulis	Measured Sn levels in tissues: digestive gland, gonad, adductor muscle, "remainder", freeze dried, Analyzed by optical emission spectrometry	

	Newport Harbor	Harbor Beach	Royal Palms	Other
Dig Gl.	3.6	1.4	1.2	1.5
Gonad	5.4	<0.3	3.4	<0.4
Muscle	<0.5	<0.7	<0.4	<0.5
Remainder	3.4	<0.5	<0.3	<0.8

(mg Sn/kg dry wt)

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